EVALUATION OF POTATO PSYLLID, *BACTERICERA COCKERELLI* (ŠULC) (HEMIPTERA: TRIOZIDAE), HOST PREFERENCES, ADAPTATION, BEHAVIOR, AND TRANSMISSION OF '*CANDIDATUS* LIBERIBACTER SOLANACEARUM' AMONG WILD AND CULTIVATED SOLANACEOUS HOSTS IN THE LOWER RIO GRANDE VALLEY OF TEXAS

A Dissertation

by

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ABSTRACT

Host plant preferences of the potato psyllid *B. cockerelli* among wild and cultivated solanaceous hosts in the Lower Rio Grande Valley of Texas, and transmission of the endosymbiotic bacterial pathogen, '*Candidatus* Liberibacter solanacearum' (Lso) were evaluated. Settling and ovipositional behavior of *B. cockerelli* was studied to determine preference for potato, tomato, pepper, eggplant and silverleaf nightshade (SLN) hosts. Results of field testing indicate resident *B. cockerelli* preferred potato and tomato equally for settling and oviposition, moving to pepper, eggplant and SLN only in the absence of potato and tomato. However, under laboratory conditions *B. cockerelli* adults preferred eggplant, pepper and potato equally, and more than tomato and SLN. Based on psyllid abundance, *B. cockerelli* were more active during the morning and less active during the afternoon. Preference for larger hosts in terms of size was exhibited, irrespective of the host. Growth and survival of *B. cockerelli* was better on potato than SLN. Lso-infectivity influenced nymphal survivorship and Lso-free individuals survived better than Lso-infective on both potato and SLN. Contrary to our hypothesis and published literature, psyllids preferred uninfected hosts and, in most cases, did not exhibit any preference for Lso-infected or uninfected potato, tomato or pepper. Results from field studies demonstrated that significantly more resident psyllids settled on uninfected potato plants than Lso-infected plants. Although previous results indicate the importance of olfactory cues to guide psyllid orientation to hosts, our results demonstrate that psyllids more likely use visual cues, preferring healthy and vigorous instead of sick

and dying hosts. *B. cockerelli* acquired Lso from infected SLN, becoming infective within two weeks and transmitted Lso back to potato. It remains unclear if SLN retains Lso after exposure to temperatures routinely >35˚C. Findings from this study will lead to useful information that can be used in a attract-and-kill scenario by attracting *B. cockerelli* adults to preferred hosts that can be used as a trap crop near potato fields. Results further highlight mechanisms that psyllids adopt in making choices for preferred hosts and opens up avenues for establishing host preference study protocols.

DEDICATION

I dedicate this dissertation

to my loving mother

for her constant support, advice and unconditional love.

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Bless the Lord O my soul, Bless His holy name;
Bless the Lord O my soul, and forget not all His benefits;
- Psalms 103:1,2

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NOMENCLATURE

Bactericera cockerelli	Potato psyllid/psyllid
Lso	'*Candidatus* Liberibacter solanacearum', bacterial plant pathogen
Las	'*Candidatus* Liberibacter asiaticus'
LRGV	Lower Rio Grande Valley
RCBD	Randomized complete block design
Hot colony	Psyllids harboring Lso
Cold colony	Psyllids free of Lso
ACP	Asian citrus psyllid

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CHAPTER I

INTRODUCTION AND LITERATURE REVIEW

INTRODUCTION

The potato psyllid, *Bactericera cockerelli* (Sulc) (Hemiptera: Triozidae), is reported to be a serious pest of several cultivated solanaceous plants, including potatoes (*Solanum tuberosum*), in the central and Western US (Cranshaw 1994, Jackson et al. 2009) and recently in the Pacific Northwest (Hamm et al. 2011). *B. cockerelli* is responsible for causing potato zebra chip (ZC) disease, a serious disorder of potatoes that has resulted in millions of dollars in losses to the potato industry (Munyaneza et al. 2007a,b). Liefting et al. (2008) reported an association between the phloem-restricted bacterium, '*Candidatus* Liberibacter solanacearum' (Lso) and ZC. Typical symptoms of potato ZC include yellowing and curling of foliage, stunted growth, formation of aerial tubers, shortened and thickened internodes, leaf scorching, reduced tuber size and yield, and early plant death (Secor and Rivera-Varas 2004; Munyaneza et al. 2007a,b; Sengoda et al. 2010). Belowground, ZC is characterized by the presence of collapsed and necrotic stolons, and browning of internal vascular tissues, which, upon frying, exhibits dark brown streaks, hence the term 'zebra chip'.

ZC was first observed around 1994 in potato crops near Saltillo in Mexico (Secor and Rivera-Varas 2004). In the US, ZC was first observed in cultivated potatoes near Pearsall and the Lower Rio Grande Valley (LRGV) areas of Texas in 2000, and has

since been detected in other states (Crosslin et al. 2010). As a direct pest, *B. cockerelli* causes significant reduction in potato yields and quality (Munyaneza et al. 2008) and over 50% loss on fresh market tomatoes (Liu et al. 2006a). When infested with psyllids, tomato (*Solanum lycopersicum*) plants exhibit chlorosis, severe stunting (due to shortened internodes) and reduced yields (*http://cisr.ucr.edu/potato_psyllid.html*).

In addition to potato and tomato, potato psyllids also feed and reproduce on eggplant (*Solanum melongena*) and pepper (*Capsicum* spp.) (Yang and Liu 2009). Although several researchers (Knowlton and Janes 1931, Pletch 1947, Wallis 1955, Yang et al. 2010a) have studied the biology of potato psyllids on cultivated solanaceous hosts, information is lacking on how these data translate into practical pest management applications. Several non-cultivated wild solanaceous plants have also been reported as alternative hosts of *B. cockerelli* (Wallis 1955) and Lso (Wen et al. 2009, Henne et al. 2010a). However, knowledge is lacking about perennial wild solanaceous hosts present in the LRGV that possibly serve as reservoir hosts, thereby enabling persistence of both the psyllid and pathogen in the absence of a potato crop.

Control of potato psyllid is complicated because psyllid populations can migrate into potato fields from distant sources (Romney 1939, Pletch 1947, Cranshaw 1994). Cultural methods such as trap cropping can be effectively incorporated into an integrated pest management (IPM) system. To be effective, a trap crop must be far more attractive to the pest, either as a food source or oviposition site, than the main crop. The significance of this dissertation study originates from the concept that alternative hosts of the potato psyllid can be exploited as trap crops near cultivated potato fields to attract,

concentrate, and kill them, but only if the trap crop is highly or at least equally favored from among the different hosts tested.

Although primarily a pest of solanaceous plants, reports dating back to the 1930's indicate that the potato psyllid can oviposit and complete development on a wide range of cultivated and wild host plants (Crawford 1914, Essig 1917, Knowlton and Thomas 1934, Pletch 1947, Wallis 1955). The four commonly cultivated solanaceous hosts of the psyllid are potato, tomato, eggplant and pepper. The biology and life history of *B. cockerelli* have been studied on potato (Yang et al. 2010a), tomato (Yang et al. 2013), and pepper and eggplant (Yang and Liu 2009) but the symptoms and epidemiology of ZC on these hosts have not yet been fully investigated. Several cultivars of potato and tomato have been tested for their resistance/susceptibility to ZC (Liu and Trumble 2004, Miller et al. 2010, Pierson et al. 2010, Butler et al. 2011). However, because the pathogen is transmitted to the host via the insect vector it cannot be determined whether resistance/susceptibility is a function of reduced insect transmission of Lso or reduced symptom development in response to insect feeding and/or the presence of the pathogen. (Further studies already in progress, but not part of this dissertation, include retesting several varieties of these solanaceous hosts for their physiological reactions to the psyllid and Lso).

Henne et al. (2010a) emphasized investigations into studies on the importance of alternative host plants, especially solanaceous weeds that may contribute to epidemiology of ZC by serving as refuges for the insect pest and reservoirs for the pathogen in the absence of cultivated species. In the LGRV, silverleaf nightshade,

Solanum elaeagnifolium (SLN), a broadleaved herbaceous and woody perennial weed native to northeast Mexico and southwestern US (Robinson et al. 1978), is widespread, abundant and commonly seen growing in proximity to cultivated potato fields (Henne and Thinakaran, unpublished). Because SLN is so abundant and difficult to eradicate, it is very important to determine whether SLN can serve as a source of primary inoculum and/or contribute to secondary infection and spread of Lso. Previously, several wild solanaceous hosts of the potato psyllid were tested for the presence of Lso under field conditions as well as after artificial inoculation with Lso by bacterialiferous psyllids under greenhouse conditions (Henne et al. 2010a). These included SLN, buffalobur nightshade (*Solanum rostrum),* and Berlandier wolfberry (*Lycium berlandierii).* It was found that SLN tested positive for Lso but was not killed by Lso infection whereas buffalobur nightshade also tested positive for Lso and the plants died quickly. In contrast, Berlandier wolfberry apparently could not be successfully infected with Lso, as the pathogen could not be detected via PCR (Henne et al. 2010a). Sengoda et al. (2010) found that annual plants that are infected with Lso are not recoverable and the pathogen can multiply for as long as the host is able to support the pathogen. However, this may not be the case with perennial wild hosts.

Insects demonstrate preferences for particular plant species, cultivars, or crop stages by responding to certain visual and olfactory cues (Hokkanen 1991). The response of the insect to these cues may be modified by the presence or absence of a pathogen in the host plant. For example, Mann et al. (2012) found an initial attraction of Asian citrus psyllid, *Diaphorina citri* adults to "*Ca*. Liberibacter asiaticus" (Las) infected citrus

plants based on volatiles these plants emitted. The possible role of Lso-induced plant responses in altering psyllid host selection behavior has not been fully investigated. Possible extensions of these studies could be development of synthetic host plant volatiles or semio-chemicals that can be used as attractants in manipulating potato psyllid behavior in more ecologically sound ways. For example, synthetic attractants could draw psyllids to an odor source where they could be concentrated, and management of these insects in smaller patches could be achieved at a lower cost to the grower.

The study reported in subsequent chapters on potato psyllid host selection was motivated by reports of preferential responses exhibited by potato psyllids for feeding (Butler et al. 2011), and ovipositing (Yang and Liu 2009) on certain hosts, and the reported preference of psyllids for Liberibacter-infected potato and citrus plants (Davis et al. 2012, Mann et al. 2012). However, direct measurement of feeding and oviposition in the field is time consuming and thus somewhat prohibitive as a tool for studying insect behavior. It is hypothesized that, for psyllids, settling behavior is likely to be an useful indicator of feeding and oviposition preference. This speculation is based on observations that settling is often accompanied by feeding. Butler et al. (2011) studied different aspects of *B. cockerelli* settling behavior such as probing, tasting, cleaning, jumping, and walking on different potato accessions. Furthermore, because *B. cockerelli* adults and immatures utilize the same food source, feeding choices made by the adult female are likely to also be good choices for progeny development. In this study, whether psyllids make choices, and the relationship between settling and oviposition

behavior is investigated. Settling behavior studies are likely to be an essential tool for synthesizing information on psyllid host selection behavior to more effectively sample and manage insect populations. For example, if psyllids display behavioral preferences this information can be used to develop better sampling and control measures. Data from monitoring and sampling surveys are useful in establishing much needed thresholds for initiating plant protection measures. Although insecticides play a pivotal role in the management of *B. cockerelli* (Nansen et al. 2010), incorporation of practices that take advantage of insect behavioral preferences could lead to more ecologically sound control methods. As mentioned above, trap crops or other attractants that concentrate insects in particular areas might be used to focus current insecticidal control practices to limited application areas. Presence of refuges within fields might also help to reduce development of insecticide resistance. To address these areas where information is lacking, field and laboratory experiments were conducted with the following objectives:

i. Evaluate preference of *B. cockerelli* to different cultivated and wild solanaceous hosts in choice and no-choice tests under field and laboratory conditions
ii. Study the life history of *B. cockerelli*, and epidemiology of ZC transmission on the common wild solanaceous host, *Solanum elaeagnifolium*
iii. Study the influence of pathogen induced plant responses to *B. cockerelli* on different solanaceous hosts under field and laboratory conditions

Application of results obtained in this study to the management of ZC disease in the LGRV is also discussed.

REVIEW OF THE LITERATURE

Host range and spread of *B. cockerelli* / ZC

The host preferences of economically important insect vectors of plant pathogens may be best studied by determining the host plants on which they feed and survive. Understanding host plant distribution patterns could provide useful information on pathogen spread and transmission by the vector. The potato psyllid, *B. cockerelli*, a vector of '*Candidatus* Liberibacter solanacearum' primarily feeds on the phloem tissues of solanaceous plants and has an extensive host range encompassing about 20 other plant families: Convolvulaceae, Asteraceace, Zygophyllaceace, Fabaceae, Brassicaceae, Chenopodiaceae, Salicaceae, Rosaceae, Polygonaceae, Pinaceae, Malvaceae, Asclepiadaceae, Amaranthaceae, Lamiaceae, Violaceae, Poaceae, Ranunculaceae, Scrophulariaceae, and Menthaceae were found to harbor one or more stages of the potato psyllid (Wallis 1955).

Besides the four main cultivated solanaceous hosts of *B. cockerelli*, potato, tomato, eggplant and pepper, several other wild and cultivated hosts also serve as breeding and overwintering hosts. Janes (1939) observed psyllids on *Lycium carolinianum, Physalis mollis* and *Solanum triquetrum*. These plants served as important wild hosts, as they were native to the US, were widespread, and locally abundant. So far, *B. cockerelli* has been reported in Texas, Oklahoma, Kansas, Nebraska, South Dakota, North Dakota, Minnesota, California, Colorado, Utah, Idaho, Montana, Wyoming and the Pacific Northwest (including Idaho, Washington, and Oregon), as well as Mexico, Central America and New Zealand (Essig 1917, Richards 1928, Binkley 1929, Knowlton

and Janes 1931, Pletch 1947, Wallis 1955, Rubio-Covarrubias et al. 2006, Gill 2006, Liefting et al. 2008, Crosslin et al. 2010, Hamm et al. 2011, Nolte et al. 2011). Breeding populations are reported to migrate from south and west Texas, southern New Mexico, Arizona, California and northern Mexico when temperatures start increasing above 32°C (Wallis 1955). Romney (1939) reported that migrating psyllid populations breed on *Lycium* sp. for several hundred miles along the Rio Grande drainage above Laredo, TX. Since *B. cockerelli* is associated with the transmission of Lso, Henne et al. (2010c) attempted to relate ZC disease patterns to *B. cockerelli* movement within potato fields, and reported that adults dispersed long distances within potato fields. These observations were based on egg and nymph counts being recorded 9m away from the point of adult release. Also, Henne et al. (2012a) suggested that ZC patterns in potato fields could be the result of primary spread of pathogenic psyllids that are infecting multiple plants in localized regions of commercial potato fields.

Sampling *B. cockerelli* populations and epidemiology of ZC disease

Monitoring insects requires prior knowledge of their biology, feeding and reproductive habits and habitats, and their activity patterns to effectively sample them. Presently, *B. cockerelli* adult populations are actively monitored with sweep nets, suction traps, and direct counts, and passively by using yellow sticky traps, pan traps and light traps. The study of insect-vectored plant diseases involves not only sampling host plants for adult and immature vectors, but also presence of the causative pathogen. Sampling also helps us understand the host selection process by adult *B. cockerelli* populations.

Potato fields need to be continuously monitored for incoming or migrating *B. cockerelli* populations, as well as to detect populations of *B. cockerelli* eggs, nymphs and adults that occur within cultivated fields, using different sampling strategies. For example, Goolsby et al. (2007) evaluated several potato psyllid sampling strategies to develop an IPM program to manage the potato psyllid and ZC in south Texas. The authors used yellow sticky cards to capture adult psyllids, and sampled leaves to determine relative abundance of potato psyllid eggs and nymphs on a weekly basis. Henne et al. (2010a) studied *B. cockerelli* adult attraction to different sticky trap colors in potato fields and found that neon-green traps attracted significantly more adult *B. cockerelli* than standard yellow sticky traps. Pletch (1947) used sweep nets (30cm diameter hoop) to sample cultivated crops for adult psyllid populations. Sweep net sampling is rapid, allows for random sampling and provides useful information about adult psyllid densities. For example, sweep net samples collected by Wallis (1955) showed a perfect negative correlation between the number of adults and the yield of potatoes. Vacuum sampling was also used to detect the presence of adult psyllids in the plant canopy, and leaves were sampled to determine egg and nymphal counts (Wallis 1955). Martini et al. (2012) proposed a leaf washing method to quantify *B. cockerelli* nymphal populations by using hot water to dislodge the nymphs from leaves, and collecting them in an organza cloth for counting.

It has been found that *B. cockerelli* have a preference for potato plants along field edges, and also tend to reside on the ventral (abaxial) sides of leaves and frequently inhabit the middle of the plant canopy (Butler and Trumble 2010) therefore, sampling

must be directed accordingly. Wallis (1955) reported that migrating psyllids landed along field edges and, as their numbers increased, they advanced inwards. Similarly, Workneh et al. (2012) conducted a ZC disease progression study and demonstrated ZC intensity to be greater along the edges than in the infields. A study conducted by Rush et al. (2010) demonstrated the importance of Lso titer variation in psyllid populations, as this could influence the amount and spread of ZC in potato fields. Henne et al. (2012a) elucidated spatial and temporal patterns of ZC infections within potato fields located in Texas Panhandle and reported that infected plants were often aggregated in clusters and numbers of infected plants within these groups increased as the disease progressed. The authors further observed that initial first foliar symptoms of ZC were expressed at potato tuber bulking stage, at which time tuber cells are expanding with accumulation of water, nutrients, and carbohydrates (Dwelle 2003). According to Wallis (1955) and Buchman et al. (2012) plants that are affected at tuber bulking stage exhibited arrested tuber growth with an associated reduction in tuber quality. Sengoda et al. (2010) found that once potato plants are infected with Lso it is not reversible. The severity of ZC depends on how early the crop is infected by the pathogen (Gao et al 2009). According to Wallis (1955) tubers that were affected by psyllid yellows produced weak plants that developed into healthy plants in the absence of any potato psyllid infestation. Henne et al. (2010b) reported that Lso-infected potato tubers also produced weak plants, and suggested that these probably do not play an important role in the epidemiology of ZC disease.

Based on geographic separation of Lso Nelson et al. (2011) reported presence of two haplotypes (A and B) to be associated with this bacterium affecting solanaceous

hosts. These haplotypes were described by single nucleotide polymorphisms (SNPs) and those occurring in Texas, Kansas and Nebraska were reported to be an overlap of both haplotypes.

Biology of *B. cockerelli*

The study of insect biology of a specific host can be useful to assess suitability of the host for feeding, oviposition, etc. Substantial work has been reported on the biology and life history of *B. cockerelli* since the 1930's and findings varied by location and host plants on which they were reared. Fitness of *B. cockerelli* is challenged by the presence of bacterial endosymbionts such as Liberibacter, *Wolbachia*, etc. (Nachappa et al. 2012, 2014) hence differences in life history parameters are observed (Yang and Liu 2009). Life table studies have been reported for potato psyllids reared on potato, tomato, eggplant and pepper, which have proven useful in analyzing population dynamics of *B. cockerelli* (Yang and Liu 2009; Yang et al. 2010a, 2013).

Potato psyllid eggs are stalked, oval-shaped and shiny yellow in color. They are deposited singly on the leaf surface, frequently along leaf edges or underside of leaves (List 1939). However, under field conditions they are predominantly found on lower surfaces of leaves (Pletch 1947). Although a maximum of 1,352 eggs was reportedly laid by a single female having a life span of 179 days (Knowlton and Janes 1931), average fecundity is reported to be 231.8 eggs per female (Abdullah 2008), and the nymphs emerge in three to nine days. Five nymphal instars are reported by Compere (1916), all of which are morphologically similar. The wing buds start developing from the third instar onwards (Pletch 1947). Nymphs change color from light yellow during early

instars to greenish blue in the later instars, and total development from nymphs into adults vary from 12 to 21 days (Knowlton and Janes 1931). Adults are cream to pale green in color on emergence and turn to gray or black when they are three to five days old (Pletch 1947). Adults begin to mate three to five days after emergence (Knowlton and Janes 1931) and they mate several times during their life span. Adult females are distinguished from males by the presence of a well-rounded short protruding ovipositor (Abdullah 2008).

Considerable differences in adult longevity have been reported, varying between 17 and 96 days (Wallis 1955). Total developmental period ranges from 25 to 33 days depending on the host on which they are reared (Yang and Liu 2009). The authors reported that *B. cockerelli* performed better on eggplant than on bell pepper, based on results that survival rates were higher and mean generation and doubling time was shorter on eggplant than on bell pepper. According to Yang et al. (2013) psyllids reared on tomato in the laboratory had greater survival, fecundity, and adult longevity compared to psyllids reared on tomato in the field. Nachappa et al. (2012) reported significant differences in seven-day female fecundity and nymphal survival of *B. cockerelli* due to the presence of Lso, which adversely affected the population growth rate.

Extreme high and low temperatures are reportedly detrimental to potato psyllid survival. Psyllid oviposition hatching and survival were reported to be ideal at 27˚C but were reduced at 32˚C (List 1939). Egg laying stopped at 35˚C. On the other hand, at least some *B. cockerelli* adults can survive subfreezing temperatures of -10°C lasting

24h, while nymphs can survive at least -15°C for 24h (Henne et al. 2010a). Alvarado et al. (2010) reported adverse effects of temperature on Lso titers and found highest mean Lso titer levels were found in potato plants grown at 28˚C. Munyaneza et al. (2012) also showed that temperatures above 32°C had detrimental effects on Lso within potato plants and Workneh et al. (2011) showed that plants grown from tubers at 15˚C had the lowest Lso titers compared to tubers grown at higher temperatures, as revealed by quantitative PCR (q PCR).

Behavioral responses and the role of olfactory/visual cues in host location by *B. cockerelli*

Host selection by insects is initially guided by olfactory and visual senses, and later by gustatory responses. Behavioral assays for studying odor detection by insects often use olfactometer or flight tunnel tests (Turlings et al. 2004). Mcindodo (1926) extracted steam distillates of solanaceous crops and identified a common odor, which he referred to as the 'potato odor'. However, differences exist in host attraction, not only among solanaceous hosts belonging to different genera, but also among different varieties/genotypes of the same species (Pierson et al. 2010). Behavior of *B. cockerelli* was studied by Liu and Trumble (2004) on tomatoes and by Butler et al. (2011) on potatoes to test differential responses of psyllids to different accessions. Of 22 potato breeding clones tested, significant differences were observed in the amount of time spent by *B. cockerelli* on the different accessions. Similarly, there were differences in Lso transmission among the potato genotypes. Pierson et al. (2010) identified certain potato accessions that did not develop ZC symptoms despite the presence of Lso-infective

potato psyllids. This lack of symptom development was suggested to be due to insect preference, plant tolerance, or both. Liu and Trumble (2004) observed that psyllids fed less, and spent more time resting on psyllid tolerant tomato cultivars compared to susceptible ones.

Transmission and translocation of Lso by *B. cockerelli*

The host plants on which *B. cockerelli* feed, may also serve as potential hosts for Lso, although Lso could occur on several other plants. Once Lso is transmitted to a host plant, plant species respond differently to the bacterium. For example, Henne et al. (2010a) found differential responses among some commonly occurring wild solanaceous hosts to infection by Lso and resultant effects on the plant. The first step in disease transmission process is for the vector to acquire the pathogen from an infected host plant/mother (horizontal and vertical transmission). Once the vector is infected, it can transmit the pathogen to another host by way of feeding. A Lso-infected psyllid is colloquially referred to as being 'hot' as long as it retains the pathogen. Presence of Lso within the psyllid varies with time and on the host on which they feed. Sengoda et al. (2013) quantified Lso titer levels in adult psyllids following different acquisition access periods from different hosts and found that titers were highest when psyllids acquired Lso from tomato than from potato. It is speculated that Lso-infected psyllids may lose the pathogen and become a 'cold' psyllid. But, mortality is almost certain for a potato plant infected with Lso (Sengoda et al. 2010). Buchman et al. (2011a) assessed Lso transmission efficiency and its effect on ZC incidence, and showed that adult *B. cockerelli* were more efficient than nymphs in transmitting Lso, and also exposing plants

to either one psyllid for 6h or 20 psyllids for 1h proved adequate to transmit Lso. In a different study, Buchman et al. (2011b) concluded that one potato psyllid was as equally damaging as 25 in causing ZC.

Information about translocation of Lso within a host plant is useful for sampling plant locations for presence of this pathogen. Lso inside host plants and psyllids can be detected by extracting its DNA (Doyle and Doyle 1990, Nachappa et al. 2011) and amplification of polymerase chain reaction (PCR). Amplification of the β-tubulin gene from potato is used as a positive control to verify quality of DNA extractions (Ravindran et al. 2011) and BC 28S for psyllid DNA extractions (Nachappa et al. 2011). Liefting et al. (2009) identified the gene sequence of the Liberibacter's 16S rDNA and since then the primer pair OA2/O12c has been used for detection of Lso. Ravindran et al. (2011) developed two new primer sets, TX 1623 F/R, that targets a conserved intergeneric region between the 16S and 23S rDNA genes and a conserved bacterial housekeeping gene, Adenylate kinase (*adk).* From Lso translocation studies conducted by Levy et al. (2011), differences in symptom development were observed between resistant and susceptible potato varieties under low insect pressure, and Lso can only be detected from developing leaves of potato and tomato beyond three weeks after infection. Detection of Lso is relatively straightforward in tomato, pepper, and eggplant, but less so with potato and SLN (Thinakaran et al. 2013). According to Wen et al. (2009) conventional PCR is often unreliable in detecting presence of Lso in plants. ZC-affected potato plants contain Lso in most of their tissues, with highest titers reported in root tissues (Crosslin et al. 2011). Crosslin et al. (2011) reported that the bacterium could be easily detected in

composite samples of Lso-infected psyllids using conventional and real time PCR with development of new and improved primers.

Management of *B. cockerelli* and ZC

Management of insect vectors of plant diseases primarily targets the vector, as economic threshold levels are not established for the vector in most cases. Being a migratory and seasonal pest, *B. cockerelli* injures plants both directly from feeding and indirectly as a vector of a plant pathogen, which warrants careful monitoring and sampling strategies for early detection. Because incidence of *B. cockerelli* and ZC progression often start from field edges and move inwards, initial control strategies should therefore target field borders and progressively move inwards as *B. cockerelli* adults are reported to move considerable distances irrespective of host plant variety, plant age and canopy architecture (Henne et al. 2010c).

Because *B. cockerelli* is an efficient plant pathogen vector that is capable of transmitting Lso within few hours of feeding (Buchman et al. 2011a), insecticidal control should provide quick knockdown of the vector to slow down ZC spread. Henne (2012) emphasized that insecticides must not only repel but also prevent/deter *B. cockerelli* adults from feeding. Because several hundreds of dollars are spent per acre for weekly rounds of spraying, it is imperative to know the presence/absence of Lso in *B. cockerelli* populations before initiating insecticidal sprays (Munyaneza 2012). Alternating different chemistries of insecticides is necessary to delay resistance in psyllid populations. Furthermore indiscriminate insecticide use leads to destruction of natural enemies, causing resurgence of pest insect populations. Insecticides commonly

used for *B. cockerelli* management in the LRGV include imidacloprid, spiromesifen, avermectin (Agrimek), spirotetramat (Movento), and dinotefuran (Goolsby et al. 2007, Henne 2012).

For practical considerations, adopted control measures should be compatible with insecticidal approaches and should work in tandem with each other to fit in an IPM model. RNA interference (RNAi) is a recent biotechnological tool that target genes responsible for vital life processes (Hail et al. 2010), and methods are being established for delivery into the potato psyllid (by injection into plants and oral acquisition) to trigger the mode of action (Wuriyaghan et al. 2011). Use of organic chemicals such as mineral oils, plant extracts, neem oil and kaolin clay has shown to deter psyllid feeding along with reduced oviposition (Peng et al. 2011, Yang et al. 2010b). Bacterial antibiotics evaluated by Henne et al. (2011) proved that the ZC infected plants could be remediated and ZC symptom expression could be delayed.

A hymenopteran parasitoid, *Tamarixia triozae*, is reported to parasitize nymphal instars of *B. cockerelli* (Pletch 1947, Wallis 1955, and Al-Jabr, 1999). Lacey et al. (2009, 2011) reported use of the fungal pathogens *Beauveria bassiana, Metarhizium anisopliae, Isaria fumosorosae* for effectively controlling *B. cockerelli* populations. Control strategies also aim to modify behavior of *B. cockerelli* adults by attracting them to a source and managing the psyllids in a limited area. To be effective, the food source should be preferred more than the main host. Munyaneza et al. (2010a) and Trevino et al. (2011) found that migrating *B. cockerelli* populations preferred early-planted potato crops. This could be due to the psyllids preferring larger hosts (in terms of leaf area and

canopy cover) when compared with later planted potato fields (which would have smaller sized plants). This was also true even when different species of solanaceous hosts were compared as reported by Thinakaran et al. (2012). Thus an early planted border crop could be used to 'attract and kill' the invading psyllid population.

Resistant/tolerant hosts play a crucial role in management of insect pests/vectors of plant pathogens in several cropping systems. Screening methods have been standardized for several insect pests to evaluate varieties, germplasm, breeding lines and accessions for resistance/susceptibility to different insects and plant pathogens under field and laboratory conditions. In the *B.* cockerelli-Lso-potato system, several commercial potato varieties have been evaluated for resistance/tolerance to ZC and it has been found that all are susceptible (Munyaneza et al. 2011). Miller et al. (2010), Munyaneza et al. (2010b), Pierson et al. (2010), and Butler et al. (2010, 2011) evaluated several potato breeding lines and germplasm accessions for tolerance to ZC. Munyaneza (2012) recommended the use of *B. cockerelli* resistant/tolerant cultivars that deter the vector from feeding thus it becomes imperative to evaluate potato varieties/lines for relative resistance against *B. cockerelli*. Breeding potato for resistance to *B. cockerelli* and ZC are underway (Miller et al. 2010, Novy et al. 2010). Studies of psyllid settling behavior are very useful and will help to differentiate resistant from susceptible hosts. Measures of feeding, oviposition, growth and development on different accessions will also help explain mechanisms of resistance/susceptibility of the different cultivars tested. Continuous and rigorous evaluation of breeding lines/germplasm resistance to *B. cockerelli* and ZC should form part of the overall psyllid IPM program.

CHAPTER II

SETTLING AND OVIPOSITIONAL BEHAVIOR OF THE POTATO PSYLLID, *BACTERICERA COCKERELLI* (SULC) (HEMIPTERA: TRIOZIDAE), ON SOLANACEOUS HOSTS UNDER FIELD CONDITIONS

INTRODUCTION

The potato psyllid *Bactericera cockerelli* (Sulc) (Hemiptera: Triozidae) is a major pest of potato in the Lower Rio Grande Valley (LRGV) of Texas. In addition to the damage caused by feeding it also transmits the bacterial plant pathogen '*Candidatus* Liberibacter solanacearum´ (Lso) the causative agent of zebra chip (ZC) disease of potato (Munyaneza et al. 2007a, Liefting et al. 2008). In US, *B. cockerelli* is the only reported vector transmitting Lso from infected to healthy plants. *Bactericera cockerelli* is primarily a pest of cultivated solanaceous crops, but alternate hosts may be important for enabling survival of both vector and pathogen in the absence of favored hosts. In the absence of solanaceous hosts, *B. cockerelli* will attack cultivated and wild hosts belonging to other plant families for feeding, reproduction or both (Wallis 1955). Pletch (1947) reported that *B. cockerelli* did not move to tomato, eggplant or pepper plants as long as potato plants were available, and wild solanaceous hosts also harbored *B. cockerelli,* but in low numbers.

Bactericera cockerelli is reported to make annual northward migrations from northern Mexico and southern Texas when daytime temperatures routinely exceed 32˚C

(Romney 1939, Pletch 1947, Wallis 1955, Rowe 1933, Liu and Trumble 2007, Goolsby et al. 2007). According to Romney (1939) and Goolsby et al. (2007), migrating populations of *B. cockerelli* in the LRGV breed on cultivated potatoes and native solanaceous hosts such as wolfberry, *Lycium* spp. and nightshade, *Solanum* spp. Wallis (1955) reported that migrating adult psyllids do prefer to oviposit on wild plants of the nightshade family before the main host establishes in the field. Cranshaw (1994) reported that the wild solanaceous plant, *Lycium carolinianum*, was commonly present around cultivated fields in southwestern Texas and served as a potential breeding host for migrating psyllid populations. List (1939) also recognized the important role of wild solanaceous hosts in supporting migrating psyllid populations. This is particularly relevant for migrating psyllid populations that harbor Lso.

Bactericera cockerelli is an effective vector of Lso, as it feeds on plant phloem tissues using piercing and sucking mouthparts. Liberibacters spp. are phloem-restricted, Gram-negative bacteria and most of them cannot be cultured in the laboratory (Bove 2006). The pathogen is able to multiply and establish itself within the vector and may be vertically transmitted (Crosslin et al. 2010). Lso has been shown to multiply within infected solanaceous crops and may be horizontally transmitted by insects feeding on infected plants (Hansen et al. 2008). Lso has been detected in wild solanaceous hosts, however the extent to which these hosts serve as reservoirs for the pathogen is still subject to investigation (Wen et al. 2009, Henne et al. 2010a). It is reported that *B. cockerelli* can acquire Lso within 8-24h of feeding on infected potato plants (Sengoda et al. 2013), and an infective psyllid can transmit Lso within 6h of feeding on uninfected

potato plants (Buchman et al. 2011a). Understanding the distribution of hosts that can harbor Lso is likely to be important for predicting sources and dispersal of primary inoculum and thus the rate and extent of disease spread. Henne et al. (2010a) emphasized investigations on vector ecology, behavior, and biology, including temperature tolerances that were also likely to be important. Little information is available concerning the distribution and abundance of wild solanaceous plants present in the LRGV that might serve as reservoir hosts of Lso, or insect behavior and utilization of these plants that will help us understand their role in the disease transmission process.

Insect behavior is a significant determinant of insect-host interactions. Insects locate their food or oviposition sources primarily by sensing visual and olfactory cues from the environment. This search process is influenced by several other factors that play a role in host selection by phytophagous insects, which include structure and physiology of host plants (Hayson and Coulson 1998), and host abundance and diversity of plant species (Lawton 1978, 1983). Fletcher and Prokopy 1991, Hendricks et al. 1991 explained that insects preferred dense foliage not only as a food attractant (Walther and Gosler 2001), but also as shelter from predation (Thomson et al 2006). Willmer (1982) reported that insects generally preferred shaded moist areas, as with *B. cockerelli,* which are commonly seen on the abaxial surface of leaves. Raghu et al. (2004) associated denser foliage with higher egg laying. Although color, shape and host plant volatiles may guide initial orientation of insects toward their hosts (Finch 1978, Chapman et al. 1981, Khan et al. 1988, Liu and Wilkins 1988) it is believed that secondary chemicals are responsible for sustaining or deterring insects from feeding on them (Hsiao 1969,

Chapman 1974, Bernays and Chapman 1977, Saxena 1986).

Whether *B. cockerelli* expresses a settling and oviposition preference behavior for different plant hosts under open field conditions was investigated using an experimental design that facilitated pair-wise comparisons of five different solanaceous hosts. Potato, tomato, pepper and eggplant that are commonly cultivated and a wild host, silverleaf nightshade, *Solanum elaeagnifolium* (SLN) were evaluated for preference by *B. cockerelli*. Preference for settling was measured by the number of resident *B. cockerelli* adults that settled on these hosts. Ovipositional preference was measured by the number of eggs laid on each host. The experiment was conducted over two field seasons to account for differences in the timing and density of insect migration. Data collected during the first season were used to inform choices regarding other potentially important variables to test in the field during the second field season including whether insect settling preference varies with patch size, time of day surveyed, or temperature.

MATERIALS AND METHODS

Biological material

Plants. The host plants tested were potato, *Solanum tuberosum* (cultivar 'Atlantic'), tomato, *Solanum lycopersicum* (cultivar 'Lance'), bellpepper, *Capsicum annuum* (cultivar 'Capistrano'), eggplant, *Solanum melongena* (cultivar 'Italian') and the locally common SLN. Potato tubers were obtained from J. W. Farms (Edinburg, TX) and seeds of tomato, eggplant, pepper, and SLN were obtained from locally propagated

stock. The above hosts were included in this study as they are the predominant cultivated and wild hosts of *B. cockerelli* in the LRGV and are also reported to be hosts for Lso.

Individual seeds of the above mentioned host plants (except potato) were planted in foam trays containing cone-shaped pots measuring 3 x 3 x 4cm filled with Metro-Mix 360 growth medium (SunGro Horticultural Distribution, Bellevue, WA). Plants were maintained in a greenhouse at 28-30˚C under natural light conditions. Potato tubers were cut in half and allowed to suberize before planting in 10cm diameter square plastic pots with potting mix added. One week-old seedlings of tomato, pepper, and eggplant were transferred to 10 cm diameter square plastic pots filled with the same potting mix. All plants were fertilized with Miracle-Gro (Scotts Miracle-Gro Products, Inc. Marysville, OH) and were watered once per week. Because potato plants in the field grew much faster than the other hosts, one week-old potato and four to five week-old seedlings of the other hosts were used to compensate for growth of potato.

Potato psyllid adult settling and oviposition

The field experiment was conducted twice between December 2012 and April 2013 at the Texas A&M AgriLife Research Center, Weslaco, TX. The soil was sandy loam and the experimental plot was prepared with a pre-plant herbicide (Dual II Magnum, Syngenta AG) for weed control (@ 150 ml/10 gallons of water) until the experimental plants established in the field. Insect settling preference was compared among potato (P), tomato (T), eggplant (E), pepper (C) and SLN (S). Four to five week-old uniformly sized plants (approximately 5-6 leaf stage) of each species (except for potato, where one week-old seedlings were used) were planted in all (15) possible paired

combinations (Fig A.1). The five 'same host' comparisons (e.g. PP, TT, CC, EE, and SS pairs) served as controls to evaluate insect settling preference under no choice conditions. The following treatment combinations were randomized in four replications.

Plant pairs were placed in close proximity to one another (30cm), and were separated from other plant pairs by at least 3m. The 15 pairs were randomized in four replications. The number of resident *B. cockerelli* adults alighting (i.e. settling) on each plant was recorded at weekly intervals, starting 20-25 days after transplant. A total of 8 observations were recorded in Trial 1 and 13 in Trial 2. To determine egg and nymph counts, leaves were sampled from middle of each plant and one compound leaf was collected from potato and tomato plants. Depending on size of the leaf, 2-3 leaves of pepper, 1-2 leaves of eggplant and 3-4 leaves of SLN were sampled to standardize for comparable leaf areas among the five different hosts. Leaves were sampled for eggs and nymphs three times in Trial 1 and twice in Trial 2. A dissecting microscope was used to count the number of *B. cockerelli* eggs, small nymphs and large nymphs present on the leaves.

This experiment was conducted using a randomized complete block design with 15 treatment pairs and four replicate blocks. Each treatment was comprised of a pair of host plants and adult settling preference was determined separately for each of the 15 host plant combinations. The response variables measured was the number of *B. cockerelli* adults that settled on each plant, which was recorded periodically commencing one month after transplant, and number of eggs and nymphs present on leaves, which were recorded two to three times during the field season. Normality of

data was examined using conditional Pearson residuals based on histograms and normal quantile plots. Analysis revealed that field counts of adults were right-skewed and data were subsequently normalized by log transformation. (Fig B.1a,b). Similar situation was encountered with count data of eggs, small nymphs, and large nymphs in both Trial 1 and 2 (Fig B.2-B.8). Homogenity of variances was indicated by plotting residual against predicted values. A statistical analysis was then performed on the transformed data (host-wise comparisons) pooled across all time points and four replications using a SAS PROC MIXED procedure, with replication as random effect. For pair-wise comparisons, difference between counts within each pair of hosts was analyzed based on repeated measures ANOVA for different dates (PROC MIXED, SAS Institute, 2013) with replication as the random factor. Data on differences in counts were found to be normally distributed with a constant variance. Although psyllid numbers varied across the different time points, the SAS PROC MIXED procedure with repeated measures estimated a pooled statistical test across all time points considering significance among hosts in each pair.

Host plant density experiment

In a separate field experiment conducted during January to April 2013, preference of resident *B. cockerelli* adults for settling was determined on plots measuring 2 x 4m ($8m^2$) containing three host densities. Two varieties of potato (i.e. 'Atlantic' and 'FL 1867') were planted in plots consisting of one, four or 16 plants, in a 2 x 3 factorial randomized complete block design with four replications. A suction trap (BioQuip products, Rancho Dominguez, CA) operated by a 12V battery with suction level

adjustment was used to sample adult *B. cockerelli* insects on each plot at weekly intervals. The adults that were aspirated from the plants at maximum suction level were counted in situ and were released immediately after counts. Data were reported as number of adults settling on each plot (i.e. number of *B. cockerelli* adults that settled on all plants in different plots) and settling by plant within a plot (i.e. total number of adults that settled in each plot divided by the number of plants in that plot). Data were analyzed using repeated measures ANOVA for the different dates with replication as random effect (PROC MIXED, SAS Institute 2013).

Time of day abundance survey

A survey was conducted to monitor abundance of resident *B. cockerelli* adults at different time points during the day. Sixteen potato plants were randomly selected (but avoiding plants along field edges) and marked in the field. Numbers of *B. cockerelli* adults that settled on the marked plants were visually counted at 8am, 12pm and 4pm with minimum disturbance. The survey was designed as a randomized complete block with each day as a block and plant as the replication factor. Number of psyllids that settled on the 16 randomly selected potato plants served as response variables for the analysis. The plants were repeatedly sampled at three time points on multiple days during the growth period and data was analyzed based on repeated measures ANOVA (repeated counts on the marked plants) at three times of the day and different dates with plant as the random factor (PROC MIXED, SAS Institute, 2013). To determine if there was a relationship between evening counts and morning counts the following day, a

correlation analysis was performed. Relationships between temperature and counts were also tested using correlation analysis.

RESULTS

Potato psyllid settling and oviposition

A. Pair-wise comparisons. (Table A.1) Psyllid adults did not exhibit settling preference when the 'same host' pairs were compared, whereas settling preferences were exhibited when 'mixed host' pairs were compared. Statistically, among the 15 paired host plant comparisons, for the five 'same host' comparisons, no significant differences in the number of psyllids that settled on each of the paired hosts were observed in either field trials. This observation demonstrates that when offered the same host (no choice), no insect settling preference was observed. However, for all 'mixed host' combinations containing potato or tomato as one member of the pair (except the combination potato vs. tomato) potato and tomato were consistently the preferred host. For the potato vs. tomato pairing, there was a significant settling preference for potato in Trial 1, but in Trial 2 there was no significant settling preference. In both trials, both members of the pairings having pepper-eggplant, eggplant-SLN, and pepper-SLN had low settling numbers and thus no significant differences in these pairings were found.

In the field, psyllid abundance varied throughout the experiment, which influenced whether settling preference for a particular host was significant or not. ANOVA F-tests for fixed effects yielded significant evidence of differences in mean responses across the eight time points (8 observation dates) in Trial 1 and 13

(observation dates) in Trial 2 (Trial 1 $F_{7,187}$=10.66, P<0.0001; trial 2 $F_{12,292}$=11.69, P<0.0001) . There was also significant evidence of differences in the 15 host pairs tested (Trial 1 $F_{14,44.9}$=5.68, P<0.0001; Trial 2 $F_{14,45}$=4.28, P<0.0001). A weak correlation was observed between adjacent time points as indicated by the lag 1 auto correlation having a value of 0.1613 for Trial 1 and 0.1589 for Trial 2.

B. cockerelli adults were present in low numbers during the first half of both Trials 1 and 2. Numbers in both trials increased as the season progressed and declined at the end of each trial (Fig A.2 and A.3). Accordingly, no significant (P>0.05) differences were observed among the different host pairs in the first half of Trials 1 and 2. Significant (P<0.05) settling preferences were observed during the second half of Trial 1 for potato and for both potato and tomato in Trial 2.

B. Host-wise comparisons. - ***Adults*** **(**Fig A.4 and A.5) - When data were pooled across the entire field season, host settling preferences were clearly evident. In field Trial 1, potato had significantly (Pr>|t| 0.0083) more *B. cockerelli* adults than tomato and both potato and tomato had significantly more *B. cockerelli* than all other hosts. The number of adults that settled on pepper did not differ significantly from eggplant (Pr>|t| 0.2937) or SLN (Pr>|t| 0.8658). In Trial 2, potato had significantly more *B. cockerelli* adults than tomato and tomato was not significantly different from pepper. Eggplant had much fewer (17.71 ± 3.34 adults/plant) across all observations and was significantly different from SLN (4.83 ± 1.08 adults/plant). ***Eggs –*** In Trial 1, a significantly greater number of eggs (211.13 ± 19.41 eggs/compound leaf) was laid on potato compared to tomato (69.46 ± 7.65). The number of eggs laid on tomato was not significantly different from

eggplant (43.75 ± 4.85) or pepper (43.08 ± 3.49). Egg numbers on these three hosts were significantly different from SLN (30.54 ± 5.67). The number of eggs laid on potato, tomato and eggplant were not significantly different in Trial 2. ***Small nymphs –*** in Trial 1, potato had significantly more small nymphs than tomato and pepper, whereas the number of small nymphs was not significantly different on potato and tomato in Trial 2. Pepper and eggplant had similar numbers of small nymphs in both trials. SLN had the lowest number of small nymphs. A similar trend was also observed with large nymphs.

Host plant density experiment

Data analysis revealed that psyllids settled on both 'Atlantic' and 'FL 1867' equally, as indicated by ANOVA F-tests, which showed there was no significant difference in the number of adults that were sampled on the two potato varieties, 'Atlantic' or 'FL 1867' ($F_{1,43.1}$=0.39, P =0.5371). However, there were differences in psyllid settling preference for different densities of plants in a plot. More psyllids settled on plots with 16 plants as determined by ANOVA F-tests, which showed a significant effect for the three plant densities tested ($F_{2,43.1}$=36.41, P<0.0001). The number of adults that settled on the 16 plant plot was significantly higher than on both the single plant (Pr> |t| 0.0001) and the four plant plot (Pr> |t| 0.0001). Similarly, the number of adults on the four plant plot was significantly higher than on the single plant (Pr> |t| 0.0012). Thus, for every four-fold increase in the number of plants in a plot, the number of *B. cockerelli* adults doubled. However, on a per plant basis, single plant plot had significantly more mean number of adults than individual plants in the four and 16 plant plot (Table A.2).

Time of day abundance

Insects settling appeared to vary with time of day with highest settling at mid-day and lower in the morning and late afternoon as determined from the comparison of number of adults counted on the plants at different census periods ($F_{2,334}$=17.29, P <0.0001). Counts on the larger field study were recorded between 8am and 12pm. Differences in counts due to adult activity between 8am and 12pm were accounted for by recording observations replication-wise. The data also showed a strong correlation between the different time points (30 observations, i.e., 10 days with three observations/day) as indicated by lag 1 autocorrelation (0.8060). Most *B. cockerelli* adults were observed during the noon time period, which was significantly different from both the 8am and 4pm observations (Table A.2). However, observations at 8am were not significantly different from observations at 4pm. Adult counts at 4pm the previous day correlated very well with counts the following morning (AR= 0.85025). There was a large variation in counts at any given temperature and it was observed that only 2% of the variation in the counts was explained by temperature within the range of 10-32˚C.

DISCUSSION

The number of *B. cockerelli* adults that settled on host pairs of the same species were not significantly different, indicating that settling did not occur by chance but settling preference was exhibited for certain hosts over the others. Furthermore, they exhibited a strong preference for potato and tomato as indicated by significantly higher

numbers that settled on these two hosts compared to eggplant and pepper among the cultivated hosts, and *B. cockerelli* adults preferred all cultivated hosts to SLN. Similar results were reported by Pletch (1947) based on studies conducted in the laboratory comparing the four cultivated solanaceous hosts. *B. cockerelli* adults did not exhibit a specific host preference for egg laying. Significantly more eggs were laid on potato than other hosts in Trial 1 and on potato, tomato and eggplant possibly due to high psyllid numbers in Trial 2. Adult activity was elevated during morning hours as indicated by significantly higher numbers of adults that were settled on plants at noon. There was no difference in adult settling on potato varieties 'Altantic' and 'FL 1867' and larger plot sizes were preferred to small plot sizes.

Visual and olfactory cues commonly used by insects for host recognition, and settling, feeding and oviposition provide them with information about host suitability for feeding and for progeny development (Anderson et al. 2013). Prokopy and Owens (1983) stated that visual cues travel in all directions at the speed of light and are unaffected by weather conditions, whereas olfactory cues are primarily driven by wind direction. Populations of migrating *B. cockerelli* arrive in the LRGV around late November to mid-December, presumably selecting their preferred hosts by using cues from their environment. In the study reported here, host settling preferences of resident *B. cockerelli* were evaluated under field conditions. The host plants tested were selected to include the four major cultivated solanaceous crops and one wild host (SLN) that was reported to be a possible breeding host for *B. cockerelli* in the absence of potatoes (Binkley 1929) and which is commonly present around cultivated potato fields in the

LRGV of Texas. Although *B. cockerelli* has been reported to use other host species, most of them are not thought to be breeding hosts. Over the 2-year observation period of this study, psyllid migration inferred from population counts occurred once during the early season and apparently was not followed by additional migrations later in the season. This was determined because no psyllids were found in a field about 2km away that was not planted with any solanaceous hosts at the time of initial psyllid migration, and no psyllid activity was documented in the field later in the season after that field had been planted with potato. The data suggests that in the LGRV psyllids migrate to areas planted with solanaceous species only during a particular time at the start of the season, and subsequent inflow of adults probably does not occur.

First generation adults started emerging by late January and peaked during first week of February, approximately 5-6 weeks after the first adult *B. cockerelli* arrived in the area. A second adult emergence peak was observed during late February and early March. Thus, two distinct peaks of adult activity occurred in the LRGV during the 2012-2013 potato growing season. *B. cockerelli* populations apparently migrate from the LRGV when daytime temperatures start routinely increasing above 32˚C during the months of April and May and are not seen again until the following December, around the time of potato growing season in this region. In this study it was observed that psyllids seem to completely disappear from the LGRV from May to November. Cranshaw (1994) reported that adult psyllids migrate in the northern direction to Colorado, Nebraska, and Wyoming during May-June and peaks during July. Interspecific competition between different groups of insects sharing a common niche or

activity of natural enemies could also interfere with host settling preference although this has not been determined experimentally. No obvious changes in activity of other important phloem feeders such as whiteflies or aphids have been reported to be correlated with disappearance of psyllids in the LRGV, suggesting interspecific competition may not be an important factor.

Resident *B. cockerelli* infested both field trials starting December 2012 to February 2013 in Trial 1 and persisted until April 2013 on Trial 2. Since plants in the field were not caged, *B. cockerelli* adults had free choice to select their preferred host. Although psyllid pressure during Trial 1 (December 2012-February 2013) was lower than Trial 2 (which included locally emerging adults) it was nevertheless sufficient to discriminate between less and highly preferred hosts. Pair-wise comparisons revealed no significant differences in pairs comparing similar hosts. In all comparisons of host pairs that contained potato, potato had significantly more adults in Trial 1. In Trial 2, comparisons of host pairs with either potato or tomato had significantly higher number of adults than the other hosts, indicating a clear settling preference for potato followed by tomato. When adult count data on individual plants from all pairs were pooled and host-wise comparisons were performed, it was also found that potato was the most preferred host followed by tomato in both field trials. SLN had the fewest *B. cockerelli* adults and plant growth was much slower than in the cultivated hosts.

This study clearly depicted the population trend of *B. cockerelli* over an entire potato growing season in the LRGV of Texas. The two distinct peaks of adult activity correlated well with psyllid population trend in the area wide psyllid monitoring

program reported by Henne et al. (2013). During onset of the growing season, greater numbers of migrating *B. cockerelli* adults settled on tomato while potatoes were just sprouting, as observed in Trial 1 (Fig A.2). To ensure uniform growth of all hosts, seedlings of tomato were planted alongside sprouting potato tubers (Fig A.6a) to accommodate for the more vigorous growth of potato. Considering that ideal environmental conditions existed for potato growth compared to other hosts, it was observed that potato grew much faster and quickly gained more leaf biomass than other hosts (Fig A.6c and A.6d). Thinakaran et al. (2012) observed that, under laboratory conditions, *B. cockerelli* adults always preferred to settle on the larger of two hosts (in terms of size and leaf area) regardless of host species presented. Accordingly, it is possible that size of the plants influenced host selection process which could have had a confounding effect on the choice made by *B. cockerelli* for potato versus other hosts under field conditions. It could be the larger size of plants that attracts migrating *B. cockerelli* adults to the early planted potato crop for settling and subsequent feeding and oviposition. Hayson and Coulson (1998) reported that host settling preference by insects is also modified depending on physiology of the host plants. In this study, it was found that psyllid settling preference was not altered by flowering, but plant aging and senescence apparently induced adult psyllids to seek out alternative hosts. When potatoes started to senesce, *B. cockerelli* adults moved to tomato, eggplant, pepper and SLN. Leaf samples of SLN contained eggs, small nymphs and large nymphs indicating that SLN supports growth and development of *B. cockerelli.*

In both trials, more eggs were present on potato followed by tomato. Thus, it was observed that *B. cockerelli* adult females laid their eggs where they settled, indicating ovipositional preference did not differ from feeding (settling) preference. Cunningham (2012) reported that oviposition preference in herbivorous insects is related primarily to whether the plant species will support development of their progeny. This may be true for Lepidoptera and other holometabolous insects where the feeding habitats of immatures are very different from those of the adults. On the other hand, in case of potato psyllids, where adults and immatures share the same ecological niche, adult choice for feeding and egg laying should be similar, as was observed in this study. Goolsby et al (2007) reported that psyllids showed no settling preference for different potato cultivars. Although nymphal densities were found to be higher on 'Atlantic' than 'FL 1867', no oviposition or settling preference was reported for either of the two potato varieties tested. In this study equal numbers of *B. cockerelli* adults were aspirated on both 'Atlantic' and 'FL 1867' on all three plant densities in the host plant density experiment.

Contemporary monitoring of *B. cockerelli* populations typically includes active sampling such as sweeping, suction traps, and direct counts, passive sampling via sticky traps, pan traps and light traps, or both. Results of the present study on settling behavior assist in understanding and synthesizing this monitoring information to effectively sample psyllids. Data from monitoring and sampling surveys will also be useful in eventually establishing threshold levels for initiating plant protection measures. For example, data from the plot size experiment indicated that single plants could serve as

sentinel plants for monitoring psyllid activity. Furthermore, plant size and location could contribute to the value of using single plants as sentinel plants since plant size and proximity to field edge are positively correlated with adult activity. Workneh et al (2012) reported a significant edge effect in the settling response of potato psyllid in Texas potato fields.

Movement of *B. cockerelli* adults within cultivated potato fields was quantified by Henne et al. (2010c) by determining egg and nymphal counts at various distances from release points. Immatures were collected up to a distance of 9m from release points, and were not affected by host plant variety, plant age or canopy architecture. However, visual counts of *B. cockerelli* adults in the study reported here indicated that psyllids did not move much after settling. *B. cockerelli* adults were not easily disturbed by adverse weather conditions (i.e. rain and wind did not appear to alter adult counts) but were disturbed if they were physically handled. Counts recorded at noon (12pm) were higher and significantly different from morning (8am) and afternoon (4pm). Furthermore there was a high correlation between evening counts and first counts on the following morning. Taken together, these data imply that adults were more likely to be observed on plants at mid-day and exhibit greater movement during mornings and late afternoons. It is important to know the time of day that corresponds with peak settling if plant-based sampling methods are to be used. Based on time of day survey results, adults should ideally be sampled on plants during late morning to early afternoon to maximize sampling efforts.

Preferential host responses were exhibited by *B. cockerelli* in the field study reported here. Attraction of *B. cockerelli* to certain hosts present opportunities to evaluate preferred hosts as trap crops around potato fields. The concept of trap cropping can effectively be used whenever insects are attracted to certain hosts rather than the main crop, either as a food source, for oviposition, or both. Differences in behavioral responses of *B. cockerelli*, as reported by Butler et al. (2011), motivated the present study on host selection. Host abundance in terms of either plant or plot size was observed to be a contributing factor for preference by *B. cockerelli* adults. Wallis (1946) reported that early planted potato crops suffered the greatest damage from psyllids. Accordingly, a border of potato or tomato planted well before the main crop could serve as a trap crop (Hokkanen 1991) to attract migrating adults and thus detection and management strategies using insecticides could be concentrated in a smaller area. Presently, insecticides play a pivotal role in the management of *B. cockerelli*. However, a more rational approach would be to incorporate ecologically sound methods that work in tandem with insecticides to contain the pest and the pathogen.

CHAPTER III

SETTLING AND OVIPOSITIONAL BEHAVIOR OF THE POTATO PSYLLID, *BACTERICERA COCKERELLI* (ŠULC) (HEMIPTERA: TRIOZIDAE), ON SOLANACEOUS HOSTS UNDER LABORATORY CONDITIONS

INTRODUCTION

The potato psyllid, *Bactericera cockerelli* (Šulc) (Hemiptera: Triozidae), is a phloem-feeding insect that is a serious pest of several cultivated solanaceous crops. It also serves as the primary vector of the phloem-restricted bacterium, '*Candidatus* Liberibacter solanacearum' (Lso), the causative agent of zebra chip (ZC) disease of potato (Munyaneza et al. 2007b, Liefting et al. 2009a). ZC was first reported in US from potato fields in southern Texas (Munyaneza et al. 2007a, b; Secor et al. 2009). Subsequently ZC has been reported from New Mexico, Arizona, Nevada, the Pacific northwest (Munyaneza et al. 2007a, b; Secor et al. 2009; Crosslin et al. 2010; Hamm et al. 2011) and New Zealand (Gill 2006, Liefting et al. 2009a, Thomas et al. 2011). Although primarily a pest of solanaceous crops, *B. cockerelli* is reported on nearly 20 plant families that can host one or more stages of this insect: Convolvulaceae, Asteraceace, Zygophyllaceace, Fabaceae, Brassicaceae, Chenopodiaceae, Salicaceae, Rosaceae, Polygonaceae, Pinaceae, Malvaceae, Asclepiadaceae, Amaranthaceae, Lamiaceae, Violaceae, Poaceae, Ranunculaceae, Scrophulariaceae, and Menthaceae (Wallis 1955). Of the cultivated solanaceous hosts of *B. cockerelli* in the Lower Rio

Grande Valley (LRGV) of Texas, potato, tomato, eggplant and pepper are predominantly grown. In addition to these cultivated hosts, several wild solanaceous hosts such as *Lycium* spp. and *Solanum* spp. are also reported to serve as breeding hosts (Binkley 1929, Pletch 1947, Wallis 1955).

Studying the distribution of wild and cultivated solanaceous host plants will likely provide useful epidemiological information related to migratory patterns, potential distribution, and abundance of *B. cockerelli*. So far, *B. cockerelli* has been reported from Texas, Oklahoma, Kansas, Nebraska, South Dakota, North Dakota, Minnesota, California, Colorado, Utah, Montana, Wyoming and the Pacific Northwest (Idaho, Washington and Oregon), as well as Mexico, Central America and New Zealand (Essig 1917, Richards 1928, Binkley 1929, Knowlton and Janes 1931, Pletch 1947, Wallis 1955, Rubio-Covarrubias et al. 2006, Gill 2006, Liefting et al. 2008, Crosslin et al. 2010, Hamm et al. 2011, Nolte et al. 2011). The *B. cockerelli* populations in different geographic locations have been categorized as belonging to three genetically distinct populations now referred to as a Western biotype comprising psyllids from California, a Central biotype comprising psyllids originating from Mexico and Texas, and a Northwestern biotype comprising psyllids from Washington, Idaho and Oregon (Liu et al. 2006b; Liu and Trumble 2007; Hamm et al. 2011; Nolte et al. 2011; Crosslin et al. 2012 a, b; Rondon et al. 2012). Due to their genetic differences, psyllids may have regionally specific food choices that influence spread of ZC in their local habitats. Thus, host selection by an insect vector has important implications for disease epidemiology. Because several non-cultivated weed hosts have been reported as alternate hosts of *B.*

cockerelli (Wallis 1955) and Lso (Henne et al. 2010a), the role of alternate host plant epidemiology in the disease transmission process of insect vectored plant diseases was emphasized by Henne et al. (2010a).

In chapter II, host plant preference of *B. cockerelli* was studied under field conditions to examine how resident psyllids choose hosts for settling, feeding and/or oviposition during the potato growing season. Potato was found to be the most preferred host, followed by tomato. When potato plants began senescing, psyllids moved to eggplant, pepper and SLN for refuge. In this study, the same field experiments were repeated under laboratory conditions whereby Lso-infected *B. cockerelli* adults were released into cages to evaluate preference by laboratory-cultured psyllids when offered only a choice between two hosts. Similar to the field study it was observed that *B. cockerelli* adults preferred cultivated solanaceous hosts potato, tomato, eggplant and pepper to the wild non-cultivated host SLN, although order of preference among cultivated hosts varied somewhat between studies. SLN was always the least preferred.

An alternate host that is highly or at least equally favored from among the different hosts tested can be exploited as an attract-and-kill trap crop. To supplement the results of field testing in chapter II, responses of *B. cockerelli* for settling and oviposition on different solanaceous hosts under laboratory conditions was evaluated. Therefore, the objectives of this study were to evaluate settling, oviposition, and pre-adaptation behavior of *B. cockerelli* adults on wild and cultivated solanaceous hosts in paired choice tests under controlled conditions.

MATERIALS AND METHODS

Biological material

A. Plants. The host plants used were potato, *Solanum tuberosum* (cultivar 'Atlantic'), tomato, *Solanum lycopersicum* (cultivar 'Lance'), bellpepper, *Capsicum annuum* (cultivar 'Capistrano'), eggplant, *Solanum melongena* (cultivar 'Italian') and the locally common silverleaf nightshade, *Solanum elaeagnifolium* (SLN). Potato tubers were obtained from J. W. Farms (Edinburg, TX) and seeds of other hosts were obtained from locally propagated sources. The above hosts were included in the study as they are the predominant cultivated and wild hosts in the LRGV.

Individual seeds of these plants were planted in foam trays containing cone-shaped pots measuring 3 x 3 x 4cm filled with Metro-Mix 360 growth medium (SunGro Horticultural Distribution, Bellevue, WA) and maintained in a greenhouse at 28-30˚C under natural light conditions. Potato tubers were cut in half, allowed to suberize before planting in 10cm diameter square plastic pots with potting mix added. One week-old seedlings of tomato, pepper, eggplant, and SLN were transplanted to 10cm diameter square plastic pots filled with the same potting mix. Plants were fertilized with Miracle-Gro (Scotts Miracle-Gro Products, Inc. Marysville, OH) once every week and watered three times a week. Four to five week-old plants of potato, tomato, eggplant, pepper and SLN of uniform size were used in the lab experiments.

B. Insects. *B. cockerelli* adults were originally collected from a potato field at the Texas A&M AgriLife Experiment Station at Weslaco, TX in May 2006 and were maintained in an insectary on potato. They were subsequently reared on tomato, pepper

and eggplant after September 2007. Starting December 2011 separate *B. cockerelli* colonies were established on all five hosts, including SLN. Psyllids were continuously reared for several generations on these hosts in BugDorm cages (catalog # 1462W 60 x 60 x 60cm rearing cage, BioQuip Products, Rancho Dominguez, CA) in an insectary maintained at 25-27˚C, 65-70 % RH, and a photoperiod of 16:8 (L:D)h. Adult *B. cockerelli* used for experimental releases were obtained from a pooled population representing all five different colonies to avoid any confounding effects due to pre-adaptation. Test psyllids were then randomly selected from the pooled population. *Bactericera cockerelli* adults for experimental release were not sexed. However, periodical sampling of Lso-infected colonies revealed a female:male ratio of about 0.42:0.58.

Settling and oviposition of *B. cockerelli* adults-paired choice test

Four to five week-old plants of potato (P), tomato (T), eggplant (E), pepper (C), and SLN (S) of uniform size (pre-flowering) were organized into pairs in all possible comparisons (Fig A.1). The 15 host pairs were placed in individual BugDorm insect rearing cages (cage size 30 x 30 x 30cm) (BioQuip Products, Rancho Dominguez, CA) having clear plastic on one side and insect-proof mesh cloth on the remaining three sides. Thirty Lso-infected *B. cockerelli* adults were collected in blue 1000µl pipette tips. The pipette opening was plugged with cotton and psyllids therein were "starved" overnight. At the onset of experiment, the tips were placed inside each cage at a mid-point, facing upwards. The cotton plug was removed and settling response of adult *B. cockerelli* was assessed at 1, 4, 8, 24, 48 and 72h after release. Insects were released in

the cages at 7:30 am and further observations were recorded accordingly. All 15 cages as well as plants within each cage were rearranged every day to minimize location effects. The adults were removed from the cages one week after release and number of eggs laid on each host was counted. Experiments were conducted in a laboratory maintained at 26-28˚C, 65-70 % RH, and a photoperiod 16:8 (L:D)h.

The experiment was conducted according to a randomized complete block design with five replications separated by time. Each treatment had a pair of plants inside a cage that served as the experimental unit. The five host plants were paired in all possible combinations to make 15 comparisons in all. Each replication consisted of the 15 plant pair treatments. The five 'same host' comparisons (i.e. PP, TT, CC, EE, and SS pairs) served as controls to evaluate insect settling preference under no choice conditions. Difference in counts of the number of psyllids that settled (alighted) on each of the two host plants in a pair was analyzed using repeated measures ANOVA for the six time points (PROC MIXED, SAS Institute, 2013) with replication and cage nested within replication as random factors. Degrees of freedom were calculated based on the method of Satterthwaite approximation. Normality of data was examined using conditional Pearson residuals based on histogram and normal quantile plots. Homogeneity of variances was indicated by residual versus predicted values (Fig B.9). A lag1 auto correlation for the six time points was accounted within the model and a modified F-test was used to test for significant effects. The conditional Pearson residuals for differences in egg counts were normally distributed and the variances were homogeneous.

No choice oviposition

Four to five week-old potato, tomato, eggplant, pepper and SLN plants were placed in individual BugDorm insect rearing cages (cage size 30 x 30 x 30cm). Six pairs of Lso-positive adult *B. cockerelli* adults were collected and released in a similar fashion as mentioned above in the previous setup. The adults were allowed to lay eggs for one week and the number of eggs laid on each host was counted using a magnifying lens. Data were analyzed as above. The conditional Pearson residuals of egg counts were found to be normally distributed and variances were homogeneous.

Plant size comparison

During the course of the investigation, it was noted that size of the host plants appeared to affect host selection behavior *B. cockerelli* adults. With this in mind, several sets of large and small sized plants were compared for preferential selection by adult psyllids.

Two to three week-old host plants (i.e. small) were compared to five to six week-old plants (i.e. large) of the same and different host species to determine whether potato psyllids had a selective preference for small or large plants in a two-choice comparison. Accordingly, comparisons were made between small and large tomato and potato plants. As a control, paired comparisons were made between two large plants of the same species (tomato and potato were used) to determine whether psyllids settled uniformly on both plants. Thirty *B. cockerelli* adults were collected and released in similar fashion as described above. Settling observations were recorded at 1, 4, 24, and 48h after release.

Pre-adaptation

An experiment was conducted in the laboratory to determine whether psyllids were orienting to the same plants on which they were reared. Each replication consisted of a pair of plants of similar size to avoid any confounding effects of plant size. Five to six week-old tomato and eggplant were placed inside insect proof cages (30 x 30 x 30cm). Thirty *B. cockerelli* adults (maintained for at least 50 generations on eggplant, tomato and potato) were collected, "starved" and released in a similar fashion as described in the laboratory settling experiment. Observations on settling behavior were recorded at 1, 4, 8, 24, and 72h after release of the psyllids.

For plant size comparison and pre-adaptation experiments, differences in numbers that settled on different hosts were analyzed using repeated measures ANOVA for the different time points (PROC MIXED, SAS Institute, 2013) with replication and cage nested within replication as random factors. The residuals did not indicate any deviation from normality and variances were homogeneous (Fig B.10).

Starvation test

To determine how long psyllids can be safely starved without inducing mortality, an experiment was conducted to assess mortality of starved male and female *B. cockerelli* adults over time. Here, adult male and female *B. cockerelli* were collected separately into individual glass vials (15 adults/vial) and then starved until dead. Ten such vials (replications) for each sex were maintained. Observations were initiated 8h after starvation and number of dead insects was recorded periodically.

RESULTS

Settling and oviposition of *B. cockerelli* adults

This experiment provided an opportunity to follow adult psyllid settling behavior from time of release until the experiment was terminated at 72h. Immediately following release, the psyllids crawled onto the sides of the cages and quickly moved to settle on the plants. During the first hour, from 20-60% released psyllids settled on host plants and percentage of adults settling increased thereafter. A faster settling response was observed on eggplants (about 30%) 1h after release followed by potato and pepper (Table A.3). After one hour, *B. cockerelli* remained settled on the preferred of two hosts provided. About 50-80% of the psyllids settled by 4h and increased steadily from 4 to 8h after release. The 24h counts were only slightly different from the 8h counts. A decrease in percent settling was observed at 48h after release and remained more or less constant until 72h.

No significant differences (P> 0.05) were found in percentages of psyllids settled on either plant at any of the six time points for the five 'same host' comparisons, (ie. potato vs. potato, tomato vs. tomato, etc., Table A.3). Results of paired host comparisons revealed no significant effect of time ($F_{5,157}$=0.80, Pr>F=0.5511) but a significant effect of host pairs ($F_{14,62}$=2.68, Pr>F=0.0040). The lag1 autocorrelation value of 0.7245 indicated a strong correlation between the different time points. For most 'mixed host' comparisons, significant differences in number of adults that settled on the two plants at one or more time points indicated a settling preference. In all comparisons that involved eggplant in a mixed pair, eggplant was preferred to all other hosts, but not significantly

more than potato and pepper. Similarly, potato was greatly preferred to all other hosts except eggplant. SLN was least preferred under both field (in chapter II) and laboratory conditions. As there was no significant effect of time, data across all six time points were pooled. Significant differences were observed in the following host pairs: Pepper vs. tomato (pepper 2.19x more psyllids than tomato), eggplant vs. SLN (eggplant 2.85x more psyllids than SLN), tomato vs. eggplant (eggplant 4.05x more psyllids than tomato), and SLN vs. potato (potato 2.04x more psyllids than SLN).

Ovipositional preferences of adult *B. cockerelli*

Adult *B. cockerelli* females laid eggs equally on all 'same host' pairs (Table A.4). However there were significant ($F_{14,56}$=2.01, Pr>F= 0.0338) differences in ovipositional preferences among certain 'mixed host' pairs. In the 'pepper-tomato' pair significantly (P> |t|=0.0039) more eggs were laid on pepper, in the 'tomato-eggplant' pair eggplant had significantly (P> |t|=0.0159) more eggs, and in 'eggplant-SLN' pair, eggplant had significantly (P> |t|=0.0182) more eggs. In contrast to the choice experiment for oviposition, number of eggs laid by female psyllids on the five different hosts in a no-choice situation did not differ significantly (P>0.05).

Plant size comparison

Comparisons between host plants of similar size and of the same species served as controls to this experiment. Accordingly, when two tomato and two potato plants of the same size were compared no significant differences were found at any time point (Fig A.7a,b). When plants of same host species but of different sizes were compared, a strong preference for settling on the larger host was found for both tomato and potato

(Fig A.7c,d). However, when a large potato plant was paired with a small tomato plant, about 7-8x more adults settled on the larger potato plant. Similar preference for the larger plant was observed if tomato were the larger of the two (Fig A.8a,b). When similar sized plants of different hosts were compared (large potato vs. large tomato) a pooled estimate over all time points showed that both hosts were preferred uniformly (Fig. A.8c). There was no significant effect of time ($F_{3,38.3}$= 1.370, Pr>F=0.2673) but there was a significant effect of host pairs ($F_{6,18.1}$=18.58, Pr>F=<0.0001). Lag 1 autocorrelation value of 0.72 indicated a strong correlation in number of psyllids between adjacent time points.

Pre-adaptation experiment

Psyllids reared on potato released onto tomato and eggplant had a strong and significant (P<0.05) preference for eggplant. The same trend was found when psyllids raised on eggplant and tomato plants were released (Fig A.9). There was no significant effect of time ($F_{5,24.6}$ = 1.49, Pr>F= 0.2277), or host pair ($F_{2,9.39}$=2.28, Pr>F=0.1556). Again, the number of psyllids settling on the hosts was autocorrelated over adjacent time points (AR1=0.5052).

Starvation test

Starved adult *B. cockerelli* males outlived females. Initial female mortality was 5.43% after 8h while 100% of males were still alive. Hundred percent of females died by 56h while about 4% of the males were still alive at 56h. One hundred percent male mortality was recorded at 72h. Time to 50% mortality of females occurred at about 30h and that of males at about 34h (Fig A.10).

DISCUSSION

In the present study, settling and ovipositional behavior of the potato psyllid, *B. cockerelli* was examined under laboratory conditions following a similar field study. Adults settled nearly equally on uniformly sized plants in 'same host' pairings, suggesting that observed differences in settling preference among 'mixed host' pairings were unlikely to be the result of random behavior. However significant differences in settling preference were found when mixed host pairings were examined. Thus ranking the five hosts based on order of settling preference by *B. cockerelli* adults in the laboratory was found to be eggplant = potato = pepper >tomato > SLN. This order of preference is slightly different from what was observed in the field with a natural psyllid infestation. The order of host preference in the field experiment (chapter II) was potato > tomato > pepper > eggplant > SLN. Field observations revealed that *B. cockerelli* adults exhibited a strong preference for potato irrespective of the growth stage, except during emergence and senescence of potato. The overwhelming preference for potato over other hosts in the field could be attributed mainly to differences in host plant sizes. In the field study, growth of potato compared to other hosts was significantly greater throughout the growing season. The role of size and vigor in settling behavior is supported by observations made early in the field season, before dramatic differences in plant size were achieved, where psyllid adults settled preferentially on tomato, eggplant, pepper and SLN. However, within 2-3 weeks, potato plants had surpassed these hosts in leaf area and biomass and attracted more psyllids than the other hosts. This preference continued until potato plants senesced, at which time psyllids moved to other hosts. In

this study, the hypothesis that plant size/vigor affected insect preference was tested. The study revealed that *B. cockerelli* settled preferentially on larger plants irrespective of the solanaceous host plant, indicating that plant size/vigor was a strong determinant of settling behavior.

This study focused both on settling and ovipositional behavior of the potato psyllid as a tool for better understanding how differences in the suitability of host plants contributes to patterns of insect movement and disease epidemiology. According to Miller and Strickler (1984), settling behavior influences the pathogen transmission process and could therefore serve as an indicator of feeding. These authors reported that host 'acceptance' is evident by continued settling and feeding by the insects and differentiated host 'acceptance' from 'preference' on the basis that insects 'accepted a host' when they had no choice. Saxena (1969) pointed out that a host might be viewed as suitable for an insect on the basis of how well the insect is able to establish populations on that host. Interestingly, Cunningham (2012) reported that empirical data often do not support the belief that phytophagous insects select their feeding or oviposition sites to maximize offspring fitness. Disparity between empirical and theoretical data suggests that several factors other than host quality may be involved in host selection behavior of phytophagous insects, as reported by Stephens and Krebs (1986), Mayhew (1997), Ballabeni et al. (2001), Scheirs and De Bruyn (2002), and West and Cunningham (2002). Berdegue et al. (1998), Cronin and Abrahamson (2001), and Mayhew (2001) reported that factors such as predation, larval movement, host-plant abundance, learning, and adult feeding sites interfered with host selection process and settling behavior did not

conform to evolutionary theory since insects were found to be attracted to poor hosts or non-hosts rather than ideal ones. It is also commonly stated that phytophagous insects select their feeding or ovipositing sites to maximize offspring fitness. However, with most hemimetabolous insects, where both adults and nymphs share the same ecological niche, choice made by the adult female for feeding and/or oviposition is also likely to be ideal for nymphal growth and development as is the case with *B. cockerelli*. Supporting this hypothesis, results from field and laboratory conditions suggest that psyllids make a choice regarding selection of host plants for settling and oviposition and that host preference for both is similar.

Together, the field (chapter II) and laboratory observations suggest that settling of *B. cockerelli* adults is a good indicator of feeding and oviposition preference given that starvation quickly leads to mortality, and the mother and her offspring share the same feeding niche. In this study it was observed that insects began settling on plants immediately after release, and 60-80% of the adults settled during the first 1 and 4h, respectively, Little subsequent movement was detected. To induce psyllids to orient toward suitable host plants, the adult psyllids were starved overnight prior to their release inside cages. Although no tests were conducted to determine whether psyllids were actively feeding, empirical observations indicate that death due to starvation would have occurred within 24-48h. In most cases, the numbers of psyllids settling remained relatively constant over the 72h observation period, suggesting psyllids were actively feeding where they settled. However, in some instances observations of psyllid settling declined from 48-72h, and this decline may be attributed to natural mortality, however,

the exact percent mortality could not be estimated, as it was not possible to retrieve the dead psyllids from the cages. The observation that feeding on an acceptable host for only a few hours results in disease transmission (Buchman et al. 2011a) suggests settling/feeding also is likely to be a good predictor of disease incidence.

It is likely that psyllids combine both olfactory and visual stimuli to locate their host from a long range and at a short range may rely on tactile and gustatory stimuli to choose its preferred host. In the greenhouse, psyllid colonies were maintained on all five hosts and psyllids for cage release were collected from a mixed population of psyllids from all colonies. A pre-adaptation test further ruled out the possibility of psyllids orienting to the host plants on which they were raised. When tomato and eggplants were provided, psyllids preferred eggplant to tomatoes irrespective of the host colony the psyllids were drawn from. Ovipositional responses showed that significantly more eggs were laid on pepper and eggplant, which was a direct result of higher settling of adults on these plants. Although it is possible that other physiological factors could contribute to settling response, such as age and sex of the psyllid, the insectary reared psyllids maintained a female:male ratio of about 0.42:0.58 and random population samples from the colony were drawn to minimize possible bias. A four-choice oviposition test conducted by Pletch (1947) revealed significant differences in the number of eggs laid on host plants and that psyllids preferred potato and tomato more than pepper and eggplant. Psyllid preference for egg laying was attributed to host leaf pubescence and egg laying was not hindered by pubescent leaves of potato, tomato or eggplant. In the present study, a no-choice oviposition experiment conducted in the laboratory showed

no significant difference in number of eggs that were laid on all five hosts, including SLN. It is therefore evident psyllids demonstrated preference for one host over the other only in the presence of two dissimilar hosts ('mixed-host'). This is clearly seen from the host plant size comparisons and established that, within solanaceous hosts, there is always a preference for the larger host which could be a confounding factor when psyllids make a host choice. It is not clear how psyllids will respond in the presence of non-solanaceous hosts. Although the experiments reported here have illuminated settling response behaviors, further investigations are needed to elucidate the mechanisms that lead psyllids to make host choices. It is possible that volatiles specific to solanaceous plants may serve as an initial attractant at a distance, and further visual, tactile or gustatory stimuli may serve as a short range cue that determines acceptability of the plant species for settling and subsequent feeding and oviposition.

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CHAPTER IV

COMPARATIVE BIOLOGY AND LIFE TABLES OF '*CANDIDATUS* LIBERIBACTER SOLANACEARUM'-INFECTED AND UNINFECTED *BACTERICERA COCKERELLI* (SULC) (HEMIPTERA: TRIOZIDAE) ON POTATO AND SILVERLEAF NIGHTSHADE

INTRODUCTION

Potato (*Solanum tuberosum*) is one of the most economically important crops in the US, and its production has of late been challenged by the potato psyllid, *Bactericera cockerelli*. *Bactericera cockerelli* is responsible for transmitting the bacterial pathogen, '*Candidatus* Liberibacter solanacearum' (Lso) which is phloem-limited (Liefting et al. 2008, 2009a,b). Most Liberibacter spp. cannot be cultured in the laboratory as reported by Bove (2006). *Bactericera cockerelli* is an efficient vector of Lso that causes Zebra Chip (ZC) disease of potato (Munyaneza et al. 2007b) in the Lower Rio Grande Valley (LRGV) of Texas and is now a serious limiting factor in potato production across the Western US as well. Potato growers have incurred severe economic losses due to this insect, even to the extent of abandoning fields (Secor and Rivera-Varas 2004, Crosslin et al. 2010). ZC has been reported from various parts of the US including Texas, Kansas, Nebraska, New Mexico, California, and Colorado (Crosslin and Bester 2009, Wen et al. 2009). Recently, ZC was reported in the Pacific Northwest (Idaho, Oregon, and Washington), which is the major US potato-growing region (Crosslin et al. 2011,

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2012a,b; Rondon et al. 2012). It was also reported to occur in Mexico, Central America, and New Zealand (Pletch 1947, Wallis 1955, Rubio-Covarrubias et al. 2006, Gill 2006, Liefting et al. 2009a, Crosslin et al. 2010, Munyaneza 2010). ZC is characterized by above ground symptoms such as upward rolling and chlorosis of newly formed leaves with purple discoloration, proliferation of axillary buds, shortened and swollen internodes, and formation of aerial tubers. ZC affected tubers show vascular discoloration, concomitant with necrotic flecking of internal tissues and streaking of the medullary ray tissues which become pronounced on frying (Munyaneza 2012).

Bactericera cockerelli is reported to have an extensive host range including 20 plant families in the Solanaceae, Pinaceae, Salicaceae, Polygonaceae, Chenopodiaceae, Brassicaceae, Asteraceae, Fabaceae, Malvaceae, Amaranthaceae, Lamiaceae, Poaceae, Menthaceae, and Convolvulaceae, (Essig 1917, Knowlton and Thomas 1934, Pletch 1947, Wallis 1955, Cranshaw 1993, Butler and Trumble 2012). Members of the majority of these families are feeding but not breeding hosts. In addition to potato, *B. cockerelli* feeds and reproduces on cultivated solanaceous hosts such as tomato, (*Solanum lycopersicum*), pepper, (*Capsicum annum*), eggplant (*Solanum melongena*) and wild solanaceous hosts, including silverleaf nightshade, *Solanum elaeagnifolium* (SLN). The potato psyllid is capable of transmitting Lso to these solanaceous hosts in the process of feeding. However, the preferred host choice may vary based on their geographic separation.

Populations of *B. cockerelli* in different geographic locations of US have been reported to possess unique genetic differences, and three distinct “biotypes” based on

these differences have been recognized using genetic markers. Swisher et al. (2012) performed high resolution melting analysis of *B. cockerelli* mitochondrial cytochrome C oxidase subunit I-like gene on more than 450 psyllids collected from different geographical regions of the western US, and three unique biotypes pertaining to three geographical regions were identified. These include the Central biotype comprising psyllids originating from Mexico and Texas, the Western biotype comprising psyllids from California, and the Northwestern biotype comprising psyllids from Washington, Idaho and Oregon (Hamm et al. 2011; Nolte et al. 2011; Crosslin et al. 2012a, b; Rondon et al. 2012). There is some overlap between these populations that does not make them clearly separable. The western and central biotypes were earlier characterized using inter simple sequence repeat (ISSR), mitochondrial gene cytochrome oxidase I, and internal transcribed spacer 2 by Liu et al. (2006b) and reported to be genetically different from each other. These populations also differ in their life history traits, as reported by Liu and Trumble (2007) based on their comparison of the western and the central psyllid biotypes.

The biology and life history of *B. cockerelli* has been studied on several cultivated solanaceous hosts under varying conditions (Compere 1916; Lehman 1930; Knowlton 1933a,b; Pack 1930; Klyver 1931; Knowlton and Janes 1931; Davis 1937; Vargas-Madriz et al. 2011; Yang and Liu 2009; Yang et al. 2010, 2013). Life table studies provide essential information on development, survival, and fecundity based on a limited number of individuals. These studies have theoretical and practical applications in population ecology models (Huang and Chi 2012). Yang and Liu (2009) investigated

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the effect of eggplant and pepper on growth and development of *B. cockerelli* and determined from life history parameters that *B. cockerelli* performed better on eggplant than pepper and they emphasized the role of host nutrition in enhancing or decreasing survival of *B. cockerelli*. Different biotic and abiotic conditions can influence population dynamics of vectors. It has been shown that environmental conditions, such as temperature, play vital roles in insect development and that both vector and pathogen appear to be sensitive to high temperatures. Accordingly, 27˚C is reported as optimum for the growth and development of *B. cockerelli* (List 1939, Pletch 1947, Wallis 1955, Abdullah 2008) and 27-32˚C as optimum for Lso titer levels (Munyaneza et al. 2012). At optimum temperatures, three to five weeks are required for *B. cockerelli* to complete one generation. Temperatures above 35˚C are reported to be detrimental to both psyllids and the pathogen (Munyaneza et al. 2012). Given the importance of alternate host plants in the epidemiology and management of *B. cockerelli* and its associated pathogen, a fundamental understanding of vector biology and population dynamics in relation to its host plants is important to support applied research. The performance of *B. cockerelli* on different cultivated and wild solanaceous hosts is essential for developing a reliable pest population prediction system that supports new management strategies. Work to date on life tables of *B. cockerelli* was restricted to major cultivated solanaceous hosts, but information on the biological parameters of this pest on wild solanaceous host is in need of attention. Also, life history parameters of *B. cockerelli* should essentially be studied in relation to its associated pathogen to understand the population dynamics of this vector in its entirety. Therefore, the objective of this study was to determine the role of Lso on

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developmental and reproductive parameters of *B. cockerelli* on SLN in comparison with its main host, potato.

MATERIALS AND METHODS

Biological material

A.Plants. The host plants used in this study: potato, *Solanum tuberosum* (cultivar 'Atlantic'), and the locally common SLN. Potato tubers were obtained from J. W. Farms (Edinburg, TX) A and seeds of SLN were obtained from locally propagated sources. Potato tubers were cut in half, allowed to suberize, and planted in 10 cm square black plastic pots filled with Metro-Mix 360 growth medium (SunGro Horticultural Distribution, Bellevue, WA) in a greenhouse near Weslaco, TX and maintained at 28-30˚C under natural light conditions. Individual seeds of SLN plants were transplanted to foam trays with cone-shaped pots measuring 3 x 3 x 4cm filled with the potting mix. When the SLN seedlings were about two week-old, they were planted in square 10cm diameter plastic pots filled with the same potting mix. The plants were fertilized with Miracle-Gro (Scotts Miracle-Gro Products, Inc. Marysville, OH) once every week. All potted plants were watered three times per week. Four to five week-old plants of potato, and SLN were used in lab experiments.

B.Insects. *Lso-infective colony: B. cockerelli* adults were originally collected from a potato field at the Texas A&M AgriLife Experiment Station at Weslaco, TX in May 2006 and were continuously reared on potato. Starting in December 2011 individual colonies of *B. cockerelli* were established on SLN. The insects were

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continuously reared on SLN for several generations in 60 x 60 x 60cm BugDorm insect cages (catalog# 1462W, BioQuip Products, Rancho Dominguez, CA) in an insectary maintained at 25-27˚C, 65-70% RH, and a photoperiod of 16:8 (L:D)h. The colonies were periodically tested for Lso using conventional PCR (procedure for DNA extraction and conventional PCR is described in chapter V) and were found to be 90-100% positive for Lso. Due to vertical and horizontal transmission of Lso in psyllids a large proportion of eggs, nymphs and adults from these colonies were expected to harbor Lso.

Lso-free colony: Bactericera cockerelli adults were provided courtesy of Dr. Joseph Munyaneza (USDA-ARS, Wapato, WA) in August 2012 and were tested by conventional PCR to be free from Lso and since then were maintained on potato and SLN plants in BugDorm insect cages under identical conditions in a separate insectary separate from the Lso-infected colony insectary. Colonies were tested periodically for Lso using conventional PCR and were always 100% free of Lso. As the Lso-uninfected colony remained 100% Lso-free, all eggs, nymphs and adults obtained from this colony were assumed to be free of the pathogen.

Egg and nymphal developmental bioassay

For each set of host plants (potato, SLN) and *B. cockerelli* colony (Lso-infected, Lso-free), three male-female pairs of adults from the respective colonies were transferred to a single leaf maintained in a 20ml glass vial filled with water and held in an inverted transparent conical container (8 x 4cm diameter) with an access hole on one side for releasing the adults. The top portion of each container was modified with an organza cloth for aeration. The adults were allowed to lay eggs for a 12h period and

were then removed with an aspirator. Ten to12 such containers (units) were set up for each of the four treatments compared ('potato-hot', 'SLN-hot', 'potato-cold', 'SLN-cold'). An individual leaf with 10-15 eggs formed a cohort (eggs of about the same age laid within a 12h period). The experiment was conducted in an insectary maintained at 25-27˚C, 65-70% RH, and 16:8 (L:D)h. Observations were recorded every day for nymphal emergence (hatching of eggs) by examining individual leaves under a stereomicroscope. The number of days to egg hatch was recorded as the incubation period. The first instar nymphs that emerged were individually identified and numbered for subsequent observation and molting. Observations were continued on a daily basis on the same individuals for subsequent molting and duration of each nymphal instar. These observations were adequate to distinguish the molted nymphs based on their exuviae, and by visually examining and comparing the size of different instars. Details on methods for differentiating the nymphal instars was also reported by Compere (1916). Rudimentary wing pads were visible early on but were well developed by the fourth instar. Emerging adults were sexed according to Abdullah (2008) to obtain sex ratio on each host.

Adult longevity and fecundity

Two days prior to initiation of the experiment, fifth instar nymphs from both Lso-infected and uninfected colonies reared on potato and SLN were collected using a camel's hair brush. They were maintained on the respective hosts and observed for adult emergence. Adults were sexed within six hours of emergence, and male-female pairs were collected and released inside the transparent inverted plastic cages following the

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experimental setup described above. Potato and SLN leaves were excised under running water to avoid desiccation and individual leaves were held in 20ml glass vials filled with water. Twenty to 30 such pairs were maintained for each of the four treatment combinations ('potato-hot', 'SLN-hot', 'potato-cold', 'SLN-cold'). The leaves were examined every day, number of eggs laid recorded, and new leaves provided until the female died, at which time female longevity was recorded. If the male died prior to the female, it was replaced with a different male to aid in continued mating and subsequent oviposition. Observations were continued until the last female died.

Life-history statistics

The egg and nymphal development bioassay response variables (i.e. incubation period, nymphal duration of the first through fifth instars, and percent nymphal survival of the different instars) were analyzed as a randomized complete block design using the PROC MIXED procedure (SAS Institute, 2013) with host and Lso as class variables and cage as the random factor. Each cohort of individuals maintained within each container (cage) comprised an experimental unit. Ten to 15 cohorts were maintained for each treatment and a total of 80 individuals were observed for potato-hot, 101 for SLN-hot, 120 for potato-cold and 114 for SLN-cold. The reproductive parameters (i.e. female longevity, pre-oviposition period, oviposition period, fecundity and sex ratio) were also analyzed using the same procedure and P> |t| values were obtained following a t-test. Degrees of freedom were calculated according to the method of Satterthwaite approximation and Tukey adjusted P-values were obtained. A total of 23 pairs were observed for potato-hot, 10 for SLN-hot, 13 for potato-cold and 15 for SLN-cold for

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reproductive parameters. In all cases, the conditional Pearson residuals were normally distributed. Homogeneity of variances was indicated by the residual versus predicted plots for each of the response variables tested. However, in the egg count data, three observations were found to be extreme outliers (zero and three eggs were laid by females that had survived for 29 and 47 days respectively, and 735 eggs were laid by a single female from Lso-free colony on potato) and were eliminated from analysis as they distorted the true means. Interaction effects were also considered between the two factors tested (host and Lso) for the various response variables (egg and nymphal duration, female longevity, oviposition, and fecundity) and were analyzed as a 2 x 2 factorial using the PROC MIXED procedure (SAS Institute, 2013) with host and Lso as class variables and cage as the random effect.

Life table statistics

Life table studies were initiated using 20-30 cohorts of individuals for each of the four treatment combinations for the purpose of recording survival, development, and fecundity until death of all individuals. All life table parameters were calculated using the SAS program written by Maia et al. (2000) (SAS Institute, 2011). Only a single value was obtained for each life table parameter (i.e. intrinsic rate of natural increase, net reproductive value, mean generation time, mean doubling time and finite rate of population increase) from the raw life table data (i.e. the daily survival rate, developmental rate and fecundity) for each individual of the cohort because of practical limitations of time and labor in obtaining replicated data. Thus, in order to estimate variability of life table statistics, Jackknife and Bootstrap techniques are usually

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recommended. In this analysis the Jackknife method (Keyfitz 1977) was used for estimating the variance of life table parameters,

The life table parameters, r_m (intrinsic rate of increase) and λ (finite rate of population increase) refer to rates of increase for a population with a stable age distribution (Birch, 1948). Gross reproduction (Σm_x) refers to the average gross number of female progeny per female, per generation. The net reproductive rate (R_0) incorporates l_x and m_x to provide a measure of net female progeny per female, per generation. Mean generation time (GT), is the average period between birth of the parents to 50% net reproduction (R_0), and is a reflection of both the pre-reproductive developmental period and fecundity (m_x)

The four treatment combinations will hereafter be referred as 'potato-hot', 'SLN-hot', 'potato-cold', and 'SLN-cold'.

RESULTS

Egg incubation, development, and survival of nymphs

The biological characteristics pertaining to egg and nymphal development are presented in Table A.5, with P-values for comparing influences of host and Lso. The average incubation period of *B. cockerelli* eggs was fastest for the Lso-infected colony when reared on potato and was significantly longer on SLN compared to potato (Pr>|t|=0.0043 for the Lso-infected colony and Pr>|t|=<0.0001 for Lso-free colony). Eggs hatched from between 2-5 days after oviposition on potato but took longer (4-10 days) on SLN. Nymphal development on SLN was also prolonged as compared to

potato. On average, nymphs developed much faster on potato (mean of 16.89 ± 0.12 days for the Lso-infected colony and 20.69 ± 0.14 days for the Lso-free colony) and this was significantly different ($P<0.0001$ for both Lso-infected and uninfected) from SLN (mean of 21.87 ± 0.29 days for the Lso-infected colony and 23.33 ± 0.14 days for the Lso-free colony). Thus, nymphs from the Lso-infected colony developed faster on potato, indicating a significant effect ($P<0.0001$) of both host and pathogen. The developmental duration of Lso-infected nymphs was prolonged on SLN, ranging from 13-23 days as compared to 11-16 days for nymphs on potato, which was not significantly different from the Lso-free colony. Except for the first instar, all instars contributed to lengthening of developmental time on SLN, mainly influenced by the developmental duration of the fourth and fifth instars.

One hundred percent of Lso-free colony nymphs on potato survived and emerged as adults (Table A.6, Fig A.11), although total egg and nymphal development of the Lso-free colony was significantly ($p<0.0001$) longer than the Lso-infected colony. Survival of fifth instar nymphs was 100% in all four treatments. The mean percentage survival of first instar nymphs was 94.00 ± 3.40% for 'potato-hot', 70.61 ± 4.04% for 'SLN-hot', 91.82 ± 3.77% for 'SLN-cold', and 100% for 'potato-cold' (Table A.6). Overall nymphal survival was lowest (54.89 ± 4.70%) on 'SLN-hot', which was significantly different from 'potato-hot' (94.00 ± 3.40%) and also significantly different from 'SLN-cold' (84.67 ± 4.70%). The effect of host was found to be an important factor explaining better survival of *B. cockerelli* nymphs on potato compared to SLN. Similarly, Lso infection also influenced survival of nymphs on the different hosts, with

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Lso-free nymphs surviving better than Lso-infected immatures on SLN, although it was not significantly different on potato.

The F_1 adults that emerged were found to have a constant female:male ratio of 1:1 in three treatments (57.5 ± 3.72: 42.5 ± 3.72 for 'potato-cold', 49.25 ± 4.72 : 50.75 ± 4.72 for 'potato-hot', and 56.57 ± 5.92 : 43.43 ± 5.92 for 'SLN-hot'). However, there were significantly ($P<0.0001$) more females than males (63.18 ± 2.85: 36.82 ± 2.85) in the 'SLN-cold' colony. Sex ratio of the four colonies were accounted in estimating life table statistics.

Significant interaction effects were found to occur between the two factors (host x Lso) for egg incubation period, and developmental period of second, third and fifth instars, indicating that any conclusions about the effect of host depended on the presence or absence of Lso. However, there was no significant interaction on the overall nymphal period, and differences were explained independently by the host and Lso.

Female longevity and fecundity

On average, females from Lso-infected colony lived significantly ($df=42$, $t=3.100$, $P>|t|=0.0172$) longer on potato (41.83 days) than on SLN (25 days). There was no significant difference between the Lso-infected and uninfected colony female life span. Lso-free females laid more eggs (227.58 eggs) and had a significantly longer oviposition period (34.17 days) on potato ($P<0.0424$) as compared to 41.08 eggs and 18.17 days, respectively, on SLN. The mean pre-oviposition period was 3-5 days for all colonies, and was not significantly different between hosts (potato and SLN) or between Lso-infected and uninfected colonies.

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An interaction effect between host and Lso (host x Lso) was observed on reproductive parameters (i.e. female longevity ($F_{1,46.4}$=10.32, P=0.0024), oviposition period ($F_{1,51}$=12.06, P=0.0011), and number of eggs laid ($F_{1,51}$=11.03, P=0.0017)). For example, female longevity is a function of Lso-infection and the host that it fed. In other words, female longevity depended not only on the host but also on Lso-infection. Therefore any conclusion on effect of host depended on presence or absence of Lso.

Life table parameters

'Potato-hot' colony recorded the highest intrinsic rate of natural increase (0.15183 day^{-}1) and the highest finite rate of population increase (0.16397 day^{-}1) combined with a minimum doubling time (4.565 days), all of which were significantly different than the 'SLN-hot' colony, but not significantly different from the 'potato-cold' colony. However, the mean generation time of 27.99 days and the net reproductive rate (70.054) of 'potato-hot' colony was not significantly different from the 'SLN-hot' colony with a mean generation time of 37.75 days and net reproductive rate of 49.04 (Table A.7, Fig A.11). Gross reproduction ($\sum m_x$) was highest (174.89) for 'SLN-hot' colony which had one female that lived 73 days (the highest female longevity) and 'SLN-cold' colony recorded the lowest gross reproduction (31.03). Graphs in Fig A.12 represent age-specific survival rate (l_x), age-specific fecundity (m_x), and age-specific maternity ($l_x m_x$). The age-specific survival is the probability that a newly hatched adult will survive to age x. From the graph it is inferred that 'SLN-cold' psyllids had the shortest oviposition period and 'SLN-hot' psyllids had the longest oviposition period.

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DISCUSSION

The study reported here was undertaken to investigate differences in life history and life table parameters of Lso-infected and uninfected *B. cockerelli* colonies maintained on potato and SLN. The biology and life-history characteristics of *B. cockerelli* have previously been studied and documented since the early 1930s under laboratory and field conditions, with considerable variability in results (Knowlton and Janes 1931; Wallis 1955; Liu and Trumble 2004, 2006; Abdullah 2008; Yang and Liu 2009; Yang et al. 2010, 2013). These studies have contributed to understanding of the biology and population dynamics of this economically important insect pest on cultivated hosts. However, herein is reported for the first time biology and life history characteristics of *B. cockerelli* on a wild host, SLN, both with and without Lso.

That significant differences exist in egg incubation periods on potato when compared with SLN, irrespective of the Lso infection, indicates an influence of a host factor on egg incubation. The minimum and maximum limits for egg hatch was higher on SLN (4-10 days) than on potato (2-5 days). It is not clear what other factors could contribute to different incubation periods in a controlled environment but it nevertheless reflects a host effect.

The total developmental period from egg to adult was significantly lower (16.89 days) for 'potato-hot' colony than 'SLN-hot' colony (21.87days) indicating a possible role of host plant nutritional quality. Based on previous research by Yang and Liu (2009), it is known that host plants can significantly influence growth, development and survival of *B. cockerelli*. Suitability of a particular host will be evident based on overall

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population growth rate, which, in turn, depends on a lower development time, higher survivorship, and fecundity. Nanthagopal and Uthamasamy (1989) carried out life table studies on *Earias vitella* on four cotton species and found that the intrinsic rate of increase (r_m), finite rate of increase (λ), and weekly multiplication ($e^{rm.7}$) were maximum on the cotton variety 'Suvin' and minimum on 'K8'. The net reproductive rate (R_0) was maximum on 'MCU 9' and minimum on 'K8'. The authors reported that 'Suvin' ideally supported population growth of *E. vitella* as observed by high rates of increase compared to 'K8', which was an unsuitable host. Differences in growth rates of *E. vitella* on 'Suvin' and 'K8' were attributed to nutritional quality of the different cotton species. From a nutritional perspective, the immature developmental period is the key deciding variable that reveals differences in the host plants compared. Longer the developmental time, the more unsuitable the host would be for *B. cockerelli*. Prolonged nymphal development period on SLN was primarily influenced by the fourth and fifth instars, indicating that later instars are nutritionally more demanding/vulnerable stages in the overall growth and development of *B. cockerelli* immatures.

Significant differences in total developmental period between Lso-infected and uninfected colonies on both potato and SLN were also found. Lso-infected colonies developed faster than Lso-free colonies, indicating that Lso was not detrimental to nymphal growth and development. This may explain better performance of Lso-infected colonies in the insectary from where study insects were collected, as compared to Lso-free colonies (J. Thinakaran pers. comm.). Nachappa et al. (2012) studied the effect of Lso on biology of *B. cockerelli*, focusing on several life-history traits including seven-

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day fecundity, hatching percentage, incubation time, nymphal survival percentage, nymphal developmental time and total developmental time. Only two traits (i.e. seven-day fecundity and nymphal survival percentage) differentiated Lso-positive from Lso-negative derived isolines. Fitness, as measured by both of these traits, was significantly lower in Lso-positive isolines, suggesting that Lso negatively influenced population growth rate of *B. cockerelli* on tomato. In the present study, overall survival of *B. cockerelli* eggs and nymphs on potato was higher than on SLN, and was higher for cold than for hot colonies, both on potato and on SLN (Fig A.11). Similar results were also found with survival of *B. cockerelli* adults, confirming negative effect of Lso. Considering the reproductive potential of female psyllids, it was determined that oviposition rate was a direct function of the length of oviposition period which, in turn, was a function of female longevity, although there were some exceptions where two females that lived for 29 and 47 days laid only zero and three eggs, respectively. Although these data were biologically interesting they distorted the true means and were eliminated from data analysis. Number of eggs laid by female psyllids is reported to be highly variable (Compere 1916, Lehman 1930, Knowlton and Janes 1931, Richards and Stephens 1932, Davis 1937, Abdullah 2008, Yang and Liu 2009, Yang et al. 2013) depending on experimental conditions. Fecundities ranging from 36-720 eggs per female have been reported on different solanaceous hosts under varied conditions and were mostly dependent on female longevity. In the study reported here, a maximum of 735 eggs was laid by a Lso-free female on potato, which was substantially higher than the majority of females. Female longevity was also found to be highly variable depending

on the host plants on which they are raised. This variability of most reproductive parameters and female longevity was attributed to frequent handling of the adults to count the eggs laid, which could have resulted in early and/or enhanced mortality, resulting in a shortened oviposition period and subsequent reduction in numbers of eggs laid. In Fig A.12 it is seen that 'SLN-hot' psyllid females had the longest oviposition period and 'SLN-cold' had the shortest. One female from 'SLN-hot' lived for 73 days, which was the longest-lived adult in the study, and could be an explanation for the highest gross reproduction of 174.89 (Table A.7).

No specific peak-egg laying period was observed. Instead, eggs were laid at discrete time intervals with several bouts of egg laying by females over their entire life span (Fig A.12). Although repeated oviposition is an innate behavior initiated by the female to overcome mortality, two definite peaks of adult emergence was observed in the field study reported elsewhere in this dissertation. Munyaneza (2012) also reported continuous overlapping generations in laboratory studies. Similar data obtained from field studies will be useful for applications of stage-specific control strategies in the future.

That females from Lso-infected colony lived longer on potato than SLN, and Lso-free females laid significantly more eggs and had a significantly longer oviposition period on potato also reflects the nutritional inadequacy of SLN plants to support adult survival. Since males were not considered in this experiment, it is not known how, or if, male longevity would be affected by SLN as a food source. That the pre-oviposition period was almost constant when psyllids were reared on potato or SLN or using Lso-

infected or uninfected colony psyllids, rules out the influence of any of these factors on the time of first oviposition.

With increasing emphasis on population dynamics studies of insects, the usefulness of life tables in the field of entomology, especially with regard to insects that vector plant pathogens, is becoming more recognized. A life table explains occurrence of certain life history events for every age interval of the organisms' life, and it is useful for predicting the outcome of specific biological parameters such as death, immature development, or adult emergence in insect mass rearing programs. This allows for efficient allocation of resources to either increase or reduce insect production that may be required at any point in time.

In summary, significant differences in nymphal growth, development and survival of *B. cockerelli* were dependent on both host and Lso-infection. Lso-infectivity status significantly influenced overall nymphal survivorship, and individuals from Lso-free colony survived better than Lso-infected on both potato and SLN. However, Lso-infected *B. cockerelli* nymphs developed faster compared to Lso-free nymphs on both potato and SLN. Lso-free females lived longer on potato than SLN, and vice versa, indicating an interaction effect between host and pathogen. Life table parameters (intrinsic rate of natural increase, net reproductive rate, mean generation time, doubling time and finite rate of population increase) revealed that growth and survival were highest on potato for both Lso-infected and uninfected colonies and was significantly different on SLN compared to potato, indicating SLN as a host to be inferior. Although SLN is reported to be a host of *B. cockerelli,* the present study was initiated to

understand the potential for SLN to serve as a reservoir host of Lso and support growth and development of psyllid in the absence of potato. Influence of host was found to be more pronounced than Lso infection on the immature survival than on female performance.

Management strategies for *B. cockerelli* must be based on a solid understanding of the ecological bases of vector outbreaks and disease incidence. The spread of insect-vectored diseases is highly dependent on the biology and ecology of vector populations. Knowledge of the symbiotic bacteria present within insect vectors can provide information about disease epidemiology, as endosymbionts can influence both vector fitness and the disease transmission process. Thus, data obtained from the present investigation will help to fundamentally strengthen our understanding of ZC disease epidemiology, and implications of these results should be considered in developing future management programs for *B. cockerelli.*

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CHAPTER V

PREFERENCE OF *BACTERICERA COCKERELLI* (SULC) (HEMIPTERA: TRIOZIDAE) FOR '*CANDIDATUS* LIBERIBACTER SOLANACEARUM'-INFECTED AND UNINFECTED SOLANACEOUS HOSTS UNDER FIELD AND LABORATORY CONDITIONS, AND TRANSMISSION OF LIBERIBACTER TO A WILD SOLANACEOUS HOST

INTRODUCTION

The potato psyllid, *Bactericera cockerelli* (Šulc) (Hemiptera: Triozidae), is a major pest of potato in the Lower Rio Grande Valley (LRGV) of Texas, and a vector of the bacterial pathogen, '*Candidatus* Liberibacter solanacearum' (Lso), responsible for causing zebra chip (ZC) disease. ZC is a devastating disease of potato that was first reported in the US from Pearsall and the LRGV of Texas in 2000 (Secor and Rivera-Varas 2004, Munyaneza et al. 2007a), subsequently causing some growers to abandon their fields. Symptoms of ZC include plant stunting, leaf chlorosis, swollen internodes, proliferation of axillary buds, and formation of aerial tubers (Wallis 1955, Cranshaw 1994, Munyaneza et al. 2007b) that are very similar to psyllid yellows disease as reported by Pletch (1947), Wallis (1955), Cranshaw (1993), and Sengoda et al. (2010). ZC affected tubers show brown discoloration along the vascular ring with intermittent flecking of medullary ray tissues (Munyaneza 2012).

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Bactericera cockerelli is primarily a pest of solanaceous plants, but adults can survive on an extensive range of host families (Essig 1917, Knowlton and Thomas 1934, Pletch 1947, Wallis 1955). Damage by *B. cockerelli* was reported in the early 1900s (Richards 1928, Binkley 1929, Richards and Blood 1933, List and Daniels 1934) when concerns were raised about the possible association of a viral pathogen, which was only much later recognized to be a bacterial disease (Hansen et al. 2008; Liefting et al. 2008, 2009a,b). The combined presence of vector and pathogen has been reported on solanaceous hosts, and is becoming a limiting factor in potato production in the western US. Psyllids and Lso affect commonly cultivated solanaceous plants, such as potato, tomato, pepper, and eggplant. However, alternative hosts are important in the epidemiology and management of this vector, by enabling survival of insect and pathogen in the absence of cultivated hosts.

Lso is a phloem-restricted, Gram-negative bacteria closely related to the Liberibacters associated with 'citrus greening', transmitted by its psyllid vector (Bove 2006), and most of them are not culturable. The pathogen restricts movement of photosynthetically-derived carbohydrates to developing tubers, resulting in formation of aerial tubers due to accumulation of starch (Gao et al. 2009). The crop stage when infection occurs is important to assess crop loss. Buchman et al. (2012) reported that tuber development was arrested if plants were infected at tuber initiation stage. However, if infection occurs later, ZC symptoms are apparent in the tubers earlier than symptom development on the aerial parts.

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With vector-borne diseases in general, the mechanisms of pathogen transmission play a key role in the disease epidemiology cycle. With ZC, the transmission process starts with Lso-infected psyllids, or with psyllids acquiring the pathogen from Lso-infected plants. With the latter, the diseased plants play a significant role in the disease transmission process. After acquisition, Lso goes through a latent period where it multiplies within the vector resulting in the psyllid becoming infective and capable of transmitting the pathogen to clean plants by feeding. Buchman et al. (2011a) demonstrated that adult potato psyllids were highly efficient vectors of Lso in transmitting ZC, more so than the nymphs. It was further shown that a single Lso-infected psyllid feeding for 6h and 20 infected adult psyllids feeding for one hour can transmit Lso to uninfected plants. Lso is currently detected in the plant by conventional and real-time PCR (Liefting et al. 2008, Wen et al. 2009). Levy et al. (2011) demonstrated that Lso was detectable in the upper and middle tier leaves of tomato and potato plants, two to three weeks after exposure of plants to Lso-infected potato psyllids.

Henne et al (2010a) emphasized the importance of conducting investigations into vector ecology, temperature tolerances, sampling strategies and alternate host plant epidemiology for insect vectors of plant diseases. Understanding host plant use patterns could provide valuable information on pathogen acquisition, transmission, and disease spread by vectors. In the LRGV, both vector and ZC symptomatic potato plants can be present in abundance (Goolsby et al. 2007), underscoring the role of alternative hosts in the disease transmission process. Little is known about what role wild solanaceous plants in the LRGV play in facilitating Lso persistence in the absence of agronomically

important solanaceous hosts. Romney (1939), Wallis (1955) and Drees and Jackman (1999) reported presence of potato psyllids in South Texas on the native solanaceous host, wolfberry, *Lycium* spp. Silverleaf nightshade, *Solanum elaeagnifolium* (SLN) is a wild solanaceous plant that is commonly found growing in large patches near potato fields in the LRGV, and possibly serving as a reservoir host for both psyllid and Lso in the absence of other solanaceous hosts. Because several cultivated and wild solanaceous hosts have been reported as carriers of Lso, it is vital to consider the role of these non-cultivated hosts in the ZC disease cycle.

Lso is transmitted both vertically from mother to offspring (Hansen et al. 2008), and horizontally by feeding on Lso-infected plants. It is therefore important to determine the role of infected plants in the disease transmission process. Here it is hypothesized that psyllids may quickly become Lso-infective by feeding on ZC symptomatic plants. However, if resident psyllid populations already harbor Lso, ZC affected plants may not contribute to further disease spread. The highest percentage of Lso-infected psyllid adults found in the LRGV during the 2011-2012 monitoring season was about ten percent (Henne et al. 2012b). Thus, psyllids and plants infected with Lso play a crucial role in the epidemiology and management of ZC. Regional monitoring of potato psyllid populations has revealed considerable differences in psyllid abundance and ZC incidence across years and across regions (Goolsby et al. 2011, Henne et al. 2012b, 2013). Information on settling behavior of *B. cockerelli* adults on solanaceous hosts and whether Lso-free psyllids can transmit Lso from infected potato to SLN and back to disease-free plants are vital components towards understanding ZC disease

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epidemiology. Therefore, the objectives of this study were to evaluate: 1. preference of potato psyllids for Lso-infected and Lso-free solanaceous hosts under field and laboratory conditions, and 2. potential role of SLN as a reservoir host of Lso in the ZC disease transmission process.

MATERIALS AND METHODS

Biological material

A.Plants. Host plants used in the study were potato, *Solanum tuberosum* (cultivar 'Atlantic'), tomato, *Solanum lycopersicum* (cultivar 'Lance'), bellpepper, *Capsicum annuum* (cultivar 'Capistrano'), eggplant, *Solanum melongena* (cultivar 'Italian'), and the locally common SLN. These hosts are commonly found growing in the LRGV and are also hosts for both psyllids and Lso. Potato tubers were obtained from J. W. Farms (Edinburg, TX) and seeds of tomato, eggplant, pepper and SLN from locally propagated sources. Seeds were planted individually in cone-shaped pots measuring 3 x 3 x 4cm filled with Metro-Mix 360 growth medium (SunGro Horticultural Distribution, Bellevue, WA) and maintained in a greenhouse at 28-30˚C under natural light. Potato tubers were cut in half, allowed to suberize, and then planted in the potting mix. When seedlings were 1-2 weeks-old, they were transplanted to 10cm diameter pots. Plants were fertilized once every week and watered three times a week or as needed. Three to four week-old potato, tomato, eggplant, pepper and SLN plants of uniform size were used in laboratory experiments.

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B.Insects. *Lso-infective colony: Bactericera cockerelli* adults were originally collected from a potato field at the Texas A&M AgriLife Experiment Station at Weslaco, Texas in May 2006 and were subsequently reared on potato, tomato, pepper and eggplant. Starting December 2011 multiple colonies of *B. cockerelli* were established on SLN. The insects were continuously reared for several generations in BugDorm insect cages (BioQuip Products, Rancho Dominguez, CA) in an insectary maintained at 25-27˚C, 65-70% RH, and a photoperiod of 16:8 (L:D)h. Bacterialiferous *B. cockerelli* adults used for experiments were obtained from the respective host colonies. The colonies were periodically tested for Lso using conventional PCR and were found to be 90-100% positive for Lso.

Lso-free colony: Bactericera cockerelli adults were provided courtesy of Dr. Joseph Munyaneza (USDA-ARS, Wapato, WA), in August 2012 and were confirmed through PCR testing to be Lso-free. Since that time Lso-free psyllids were maintained on the five hosts (i.e. potato, tomato, pepper eggplant and SLN) in BugDorm cages under identical conditions in an insectary separate from the Lso-infected colony room. Colonies were tested periodically for Lso using conventional PCR and were found to be free of Lso. Lso-free adults for experimental releases were obtained from the respective colonies.

ZC transmission to host plants

Twenty-four, 3-4 week-old potato, tomato, eggplant, pepper, and SLN plants raised in a greenhouse under cages were selected for the study. Seven *B. cockerelli* adults from the Lso-infected colony were introduced into white organza bags tied to 12

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plants (the other 12 plants were left uninfected for paired testing) on a lower leaf, ensuring no psyllids could escape. One week later, the entire leaf along with psyllids and bag, was removed. Leaf midrib samples along the top tier of individual plants were tested for Lso three weeks later using the procedure described below.

Lso Testing

A.Plant DNA extraction. Total genomic DNA was extracted from plants using the CTAB (cetyltrimethylammonium bromide) method as described by Buchman et al. (2011a) with modifications. A portion of the leaf midrib (or root or stolon) measuring ~0.3g constituted a sample. Each sample was cut into small pieces and placed in a 2ml lysing matrix A tube (MP Biomedicals, Santa Ana, CA) with 1000µl of plant extraction buffer and pulverized for 4 min using a Mini Beadbeater-96 (BIOSPEC Products Inc, Bartlesville, OK). Tubes were centrifuged @12000rpm for 2min. and 300µl of the supernatant was pipetted into 1.5ml microcentrifuge tubes (Fisher Scientific Inc. Pittsburgh, PA) containing 80µl of lysozyme and incubated in a dry bath for 30min @ 37˚C. Then 500 µl of CTAB buffer was added to the homogenate after which samples were incubated for 30min @ 65˚C. Samples were brought to room temperature and 500 µl of ice-cold chloroform was added. The samples were vortexed and centrifuged @12,000rpm for 10min. The supernatant was transferred to a new 1.5ml microcentrifuge tube containing 1.3µl glycogen and 500µl of isopropanol and placed on ice or frozen overnight. The precipitated DNA pellet was washed with ice-cold 70% ethanol and centrifuged at 12,000rpm for 2min. The ethanol was drained and the pellet was air-dried at 37˚C. The DNA pellet was then eluted in 50µl nuclease-free water.

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B.Psyllid DNA extraction. Total genomic DNA was extracted from psyllids using the CTAB extraction method described in Buchman et al. (2011a) with modifications. Individual psyllids were placed in 1.5ml microcentrifuge tubes (Fischer Scientific Inc. Pittsburgh, PA) containing 500 µl of CTAB buffer and crushed using a micropestle. Samples were then incubated for 30min at 65˚C. Samples were brought to room temperature and 500µl of ice-cold chloroform was added. The samples were then vortexed and centrifuged for 3min at 12,000rpm. The supernatant was transferred to a new 1.5ml microcentrifuge tube containing 1.3µl glycogen and 500µl of isopropanol and placed on ice for 20min or in the freezer overnight. The precipitated DNA pellet was washed with ice-cold 70% ethanol and centrifuged at 12,000rpm for 2min. The ethanol was drained and the pellet air-dried at 37˚C. The DNA pellet was then eluted in 100µl of nuclease-free water.

C.Conventional PCR assay for Lso-detection in solanaceous hosts. Lso detection in plant samples was performed using Lso TX 16/23 forward and reverse primers (Ravindran et al. 2011). Amplification of ß-tubulin gene was used to indicate quality of DNA extractions with Btub1F 5′-TGATTTCCAAGGTAAGGGAGGA-3′ and Btub 1R 5′-CATGTTGCTCTCGGCTTCAG-3′. Quality of psyllid DNA was confirmed using BC 28S F/R primers.

Settling of resident *B. cockerelli* adults on three and five week-old Lso-infected and Lso-free potato plants-Field

Two field experiments were conducted during December 2012 to February 2013 and February to April 2013 to evaluate preference of resident populations of *B.*

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cockerelli adults for Lso-infected or Lso-free potato plants. Potatoes were planted two weeks apart (to enable simultaneous availability of both 3 and 5 week-old plants to resident psyllids). Tubers were planted in pairs separated by 30cm and covered with soil emergence cages (catalog # BT2007, 60 x 60 x 60cm soil emergence trap-headless insect rearing tent, BugDorm store, Megascience Co. Ltd., Taiwan) to avoid incidental infestations. Five weeks after first planting, ten Lso-infected *B. cockerelli* adults were introduced into white organza bags that were clipped onto a single mid-canopy leaf for Lso transmission. One week later, the bags along with psyllids and leaves were removed to ensure no psyllids were left inside the cages. One month later, the soil emergence cages were removed and numbers of resident *B. cockerelli* adults settling on plants was recorded at intervals until plant death or senescence.

Each trial was planted as three paired comparisons: Lso-infected/infected, Lso-infected/uninfected, and Lso-uninfected/uninfected in a randomized complete block- 2 x 3 factorial design with plant age (two levels – three and five weeks after emergence) as the first factor and Lso-infection status (three levels – pair 1 uninfected vs. uninfected, pair 2 –uninfected vs. infected, and pair 3 – infected vs. infected) as the second factor. No interactions among the factors were considered and hence the data were analyzed considering the six treatments. The experiment was replicated five times and each replication served as a blocking variable. The two field trials conducted are hereafter referred to as Trial 1 and Trial 2, for the first and the second trial, respectively.

The response variable (i.e. the settling response of *B. cockerelli* adults) was assessed throughout the entire growing season. Data were analyzed using PROC

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MIXED procedure (SAS Institute, 2013) with repeated measures ANOVA across the different time points tested. Each cage with a pair of plants formed an experimental unit (although cages were removed later) to which the treatments were applied. Analysis was performed with replication and cage nested within replication as random factors. Tests for fixed effects revealed whether or not there was a significant effect of time or pair or time x pair. The least squares means table provided information of the significance of mean differences between pairs compared using a t-test across the six treatment combinations at each of the six time points for field Trial 1 and five time points for field Trial 2. A pooled significance indicated which of the treatment pairs were significantly different across all time points pooled together. Normality of data (difference in adult numbers that settled on both hosts in each paired combination) was examined using conditional Pearson residuals based on histogram and normal quantile plots for Trial 1 and 2. Homogenity of variance was indicated by plotting residual against predicted values for each of the pairs tested (Fig B.12 and B.13).

Laboratory evaluation of *B. cockerelli* adult settling preference for three and six week-old Lso-infected and Lso-free solanaceous hosts

Lso-infected and Lso-free potato, tomato, pepper, eggplant, and SLN plants of uniform size were arranged into the following pairs:

PAIR 1 (control): neither plant infected;

PAIR 2 (treatment): reciprocal pairings of one infected and one uninfected plant;

PAIR 3 (control): both plants Lso-infected.

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Three pairs with four replications of each pair were maintained for each set of hosts. Each pair was placed in individual BugDorm insect rearing cages (cage size 30 x 30 x 30cm, BioQuip Products, Rancho Dominguez, CA). Thirty Lso-positive *B. cockerelli* adults were aspirated into pipette tips, the opening plugged with cotton, and were starved overnight prior to release in cages. The tips were then placed inside each cage from a mid-point facing upwards, and the cotton plug was removed to allow adults to disperse. Settling response of *B. cockerelli* was assessed at 1, 4, 8, 24, 48 and 72h after release and the treatment combinations compared. Cages were rearranged and plants therein switched once each day to minimize location effects. A set of ten experiments was conducted using psyllids from the Lso-infected colony with the three paired host combinations mentioned above using the following host plant stages:

1. Potato – 1, 2, and 3 weeks after Lso-infection (WAI)
2. Tomato – 3 and 6 WAI
3. Pepper – 3 and 6 WAI
4. Eggplant – 3 and 6 WAI
5. SLN – 4 WAI

A similar set of ten experiments was also conducted using Lso-free psyllids. All experiments were conducted at 26-28˚C, 65-70 % RH, and a photoperiod 16:8 (L:D)h. Each experiment was conducted in a randomized complete block design with three treatment combinations [pair 1 (control)- neither plant infected, pair 2 (treatment)- reciprocal pairings of one infected and one uninfected plant, and pair 3 (control)- both plants Lso-infected] and four replications for each treatment. Each pair of plants within a

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cage constituted an experimental unit. Differences in number of psyllids that settled on each of the two hosts in a pair was analyzed based on repeated measures ANOVA for the six time points (PROC MIXED, SAS Institute, 2013) with replication and cage nested within replication as random factors. Degrees of freedom were calculated based on the method of Satterthwaite approximation. Data from these experiments (difference in adult numbers on both hosts in a pair) was examined for normality using conditional Pearson residuals based on histogram and normal quantile plots. Homogeneity of variances was indicated by residual versus predicted values. A lag1 autocorrelation for various time points was accounted for within the model and a modified F-test was used to test for significant effects. All 20 experiments were analyzed using the same approach and all datasets were normally distributed with homogeneous variance (Fig B.14-B.23).

Lso transmission from potato to SLN and vice-versa, and retention of Lso in SLN

Four to five week-old greenhouse-grown SLN were infected with Lso using bacterialiferous *B. cockerelli* adults that were released into white organza bags tied to a lower leaf and allowed to feed for one week. Three weeks later, ten SLN plants that tested Lso-positive were placed into each of two cages. Five hundred *B. cockerelli* adults from Lso-free psyllid colony were released into each cage and allowed to feed on Lso-infected SLN. Periodically, starting three days after release, psyllid adult samples were randomly drawn from each cage and Lso presence in psyllids was detected by PCR per the procedure detailed above. Twenty Lso-infected SLN plants were planted in the field during January 2013 and covered with soil emergence cages (catalog # BT2007 60 x 60 x 60cm soil emergence trap-headless insect rearing tent, BugDorm store, Megascience

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Co. Ltd. Taiwan) to avoid incidental infestation. Samples of leaf midrib from aerial parts of the plant were collected during September 2013 and tested for presence of pathogen according to DNA extraction and PCR procedures detailed above.

RESULTS

PCR detection of Lso, and Lso-infected host symptomatology

Leaf midrib samples of all hosts were used to detect presence of Lso. Presence of the predicted 383-bp 16S-23S rDNA band using the TX 16/23 F/R primer pair was indicative of samples being Lso-positive. Leaf midrib samples of potato and SLN were tested for presence of Lso three weeks after Lso-infection. Results were not consistent with symptom expression despite having high quality DNA extraction as demonstrated by the positive control.

A. Effect of Lso on growth of potato. Four to five week-old potted potato plants (cultivar 'Atlantic') artificially inoculated with Lso for one week in the field and laboratory began exhibiting symptoms three weeks after inoculation and the plant wilted and died during or by the end of that same week. During the first two weeks, plants appeared normal and not any different from uninfected plants (Fig A.13, A.14).

B. Effect of Lso on growth of tomato. Four to five week-old tomato plants artificially inoculated with Lso, did not show any visible symptoms until four weeks after inoculation or later. From fifth week onwards plants began stunting and leaves started to twist and curl. However, symptoms were not identical on all plants infected with Lso at the same stage and time, but varied among plants. However, sixth to seventh

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week on plants began to sicken more with symptoms of yellowing and intermittent browning of foliage (Fig A.15).

C. Effect of Lso on growth of eggplant and pepper. Eggplants and peppers were very robust and resistant to infection by Lso. Neither host plant species expressed any adverse symptoms until the fifth week or later, and diseased pepper plants successfully flowered. Beyond fifth week, pepper plants began to disfigure somewhat but lacked any symptoms of yellowing or wilting, and were still alive at eight to nine weeks after Lso-infection (Fig A.16), after which time plants expressed severe symptoms and collapsed and died. However, PCR-testing for Lso revealed that they were indeed infected with Lso. In contrast, eggplants did not develop any adverse symptoms of Lso infection (Fig A.17), but plants wilted and died starting from the sixth to seventh week.

D. Effect of Lso on growth of SLN. Greenhouse grown SLN plants had a very different growth pattern from all other host plants tested, and were also very different from field grown plants. Under greenhouse and laboratory conditions, leaves of SLN plants were a lush green color and lacked any thorns, whereas field plants had silvery grey leaves with thorns/trichomes along the midrib and base of the leaf. Lso inoculated SLN plants tolerated infection without exhibiting any symptoms (Fig A.18) whatsoever until four to five weeks after infection. Although plants dropped their leaves beyond five weeks in pot-bound conditions, underground portions remained alive and sprouted by producing underground stolons.

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Settling of resident *B. cockerelli* adults on three and five week-old Lso-infected and Lso-free potato plants-Field

Upon removal of field cages it was observed that some of the Lso-infected potato plants (both 3 and 5 week-old when infected) exhibited symptoms of wilting within one month of infection. Psyllids did not prefer any host but settled uniformly on all hosts within a pair in Trial 1. Based on repeated measures MIXED model analysis, no significant differences were observed in any of the treatment comparisons in Trial 1. Psyllid abundance in Trial 1 from December 2012 to February 2013 was less compared to Trial 2 at the same location from February to April 2013 (Fig A.19). There was no significant difference in psyllid counts across the different dates in Trial 1 and neither was there any interaction between treatments and dates. The lag 1 auto correlation between observations was not strong for Trial 1 (0.3056) and very low for Trial 2 (0.1932). Psyllids preferred Lso-free plants in the uninfected vs. infected comparison (5 week-old when infected) in Trial 2. Trial 2 had much higher resident psyllid pressure compared to trial 1. The mean number of psyllid adults per plant ranged from 0-5 in Trial 1 but were from 5-18 in Trial 2 throughout the season (Fig A.19). Psyllid abundance increased starting first day of March 2013 onwards and declined by end of that month.

ANOVA F-tests for fixed effects yielded significant evidence of a difference in mean responses across the five time points in Trial 2 ($F_{4,61.9}$=3.220, Pr>F=0.0182). There was also significant evidence of differences among the six host pairs tested ($F_{5,24.2}$= 4.290, Pr>F=0.0062). There were no interaction between treatments and sample

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dates ($F_{20,61.9}$=0.750, Pr>F=0.7627). Comparing the six treatments (i.e. 2 levels of plant age at infection (3 and 5 week-old plants) and three pairs of treatment combinations for each plant age), a significant effect was found for pairs of uninfected vs. infected plants infected at five weeks of age, where more psyllids were found on uninfected plants (Table A.8). There was also a significant difference in psyllid counts on uninfected vs. uninfected plants at five weeks of age. However, no significant differences were evident in any of the treatment combinations involving three week-old infected plants.

Laboratory evaluation of *B. cockerelli* adult settling preference for three and six week-old Lso-infected and Lso-free solanaceous hosts

Results of the series of 20 experiments, ten with Lso-infected psyllids and ten with Lso-free psyllids, on the different host plants are represented in graphs (Fig A.20 - A.27) and summarized in Table A.9. In all experiments, no significant differences in adult settling were found among the control pairs (i.e. Lso-infected vs. Lso-infected and Lso-uninfected vs. Lso-uninfected), indicating equal preference for similar hosts. Significant differences in settling behavior were evident in the Lso-uninfected vs. Lso-infected treatment combination in experiments 1, 3, 4, 9, 12 and 17 (Table A.9). In all cases, significantly more psyllids settled on the Lso-free plants (Table A.9). Choices made by psyllids at different time points for each paired comparison and for all experiments are presented graphically (Fig A.20 – A.27).

A. Settling preference for potato- one, two and three weeks after Lso-inoculation (Fig A.20 and A.21). Lso-infected adult psyllids settled more frequently on uninfected than infected potato at 1, 8, 48 and 72h intervals, one week after Lso-

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infection (P=0.018, 0.045, 0.021, and 0.024 respectively) (Fig A.20b). At two and three weeks after infection a similar preference by Lso-infected psyllids for pair two was evident (Figs A.20e and A.20h). Significantly more Lso-free psyllids settled on the uninfected plant (Fig A.20e) two weeks after infection.

B. Settling preference for tomato -three and six weeks after Lso-inoculation (Fig A.22 and A.23). Lso-infected psyllids preferred uninfected tomato plants, with significant differences at 1, 48 and 72h (P=0.0062, 0.0001, 0.0004, respectively) at three weeks after infection (Fig A.22b). Lso-free psyllids initially preferred Lso-infected tomato plants at three weeks after infection, and this preference was significantly different at 1, 4, and 8h (P=0.0411, 0.0214, 0.0411, respectively). Differences were not significant after 8h (Fig A.23b).

C. Settling preference for pepper-three and six weeks after Lso-inoculation (Fig A.24 and A.25). Psyllids did not make a choice but rather settled uniformly, and no significant differences were found with any host comparisons using Lso-infected psyllids (Figs A.24a-f). Similarly, Lso-free psyllids showed no preference for either pepper host, except for a significant preference for an infected plant at 1h (P= 0.0056) (Fig A.25e).

D. Settling preference for eggplant- three and six weeks, and SLN- four weeks after Lso-inoculation (Fig A.26 and A.27). Comparing eggplant three weeks after Lso-infection, significantly more Lso-infected psyllids settled on one of the infected plant in pair 3 (infected vs. infected-Fig A.26c) at 1 and 24h (P=0.0332 and 0.0429, respectively) and no settling preference was exhibited by Lso-free psyllids (no significant difference

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was detected) However, six weeks after Lso-infection, uninfected plant in the uninfected vs. infected (pair 2) attracted significantly more Lso-infected psyllids at 4, 8, 24 and 72h (P= 0.0104, 0.0265, 0.0076, and 0.0166, respectively-Fig A.26e). Six weeks after Lso-infection, infected vs. infected (pair 3) had significantly more psyllids (Fig A.26f) at 4 and 8h (P=0.0194 and 0.0194, respectively). However, with the same set of experiments using Lso-free psyllids, settling was equal on both plants in a pair and no significant differences were observed in any of the comparisons whatsoever. A similar trend was observed using both Lso-infected and uninfected psyllids on SLN, and no preference was found as indicated by non-significant P-values (P>0.05).

Transmission and retention of Lso in SLN

Lso was not detected in Lso-free psyllids at five days after feeding on Lso-infected SLN plants (PCR amplification using the BC28S F/R primers confirmed quality of DNA extraction). One of ten psyllids tested positive for Lso two weeks later. 3/15 (20%) and 4/15 (27%) tested positive for Lso at four and six weeks respectively. Psyllids were able to acquire Lso from infected SLN plants and become infective within two weeks of feeding. Aerial portions of Lso-infected SLN planted in the field during spring 2013 were sampled for Lso in September 2013, but Lso has not been detected in leaves and stems thus far.

DISCUSSION

Elucidating interactions between insect vectors, plant pathogens, and host plants is important to fully understand the epidemiology of vector-transmitted plant diseases. In

potato and other solanaceous hosts, the main mode of Lso transmission is via the insect vector, *B. cockerelli*. Field and laboratory studies were designed to address the same hypothesis using two different approaches. The field experiment was a study of host plant age at time of Lso infection as the main factor, as opposed to the number of weeks after Lso infection being the main factor in the laboratory study. In the field study, potato plants were infected when they were three and five weeks-old and were exposed to psyllids one month after infection. In the laboratory, all plants were three to four weeks-old when they were infected with Lso but were exposed to psyllids three and six weeks after Lso-infection. It was hypothesized that psyllids use both olfactory and visual cues to detect their preferred hosts. The field and laboratory experiments were set up in a way to have sick but not dead plants from Lso-infection. When comparing Lso-infected and uninfected plants caution was exercised to maintain uniformity in plant size. This was done by using an uninfected plant of lesser age compared to the Lso-infected plant. Plants used in all laboratory experiments were in pre-flowering stage. Potato plants infected with Lso died within three weeks and accordingly observations were recorded at one, two and three weeks after Lso-infection in the laboratory. Field experiments gave a broader perspective of how resident psyllids make their choice of hosts at a long range throughout the growing season. Laboratory studies were more controlled and highly focused and recorded observations on settling response within a window of 72h.

Substantial variation in disease symptoms were observed in the field on potato plants at both three and five weeks after infection (Fig A.13). Psyllid abundance was low

(mean of 0-5 psyllids/plant) in Trial 1, but was higher (mean of 5-18 psyllids/plant) in Trial 2. In Trial 1, psyllid preferences could not be detected (revealed by no significant differences in any of the treatment pairs) probably due to low psyllid abundance. Psyllid abundance in Trial 2 was sufficiently high to allow meaningful conclusions on how psyllids selected between Lso-infected and uninfected potato plants. The very low autocorrelation in psyllid abundance for Trial 2 across different dates was due to the high variability in resident psyllid abundance between different observations. Trial 1 coincided with in-migrating psyllids, whereas Trial 2 had resident psyllid population that built up following Trial 1. When significant differences were found such as with five week-old Lso-uninfected and Lso-infected plants (Table A.8), more psyllids settled on uninfected compared to infected plants and differences were highly pronounced showing that psyllids did not prefer infected plants. Significant adult settling differences in five week-old uninfected vs. uninfected control plants could be attributed to differences in plant sizes, which could not be controlled under field conditions. Furthermore, a couple of uninfected plants died for reasons unknown.

Previous fieldwork contributed in several ways to improving the design of experiments described here. For example, in previous field and laboratory experiments (reported in chapters II and III), it was documented experimentally that size of the plant is an important factor influencing choices made by psyllids. Thus, under laboratory conditions, care was taken to overcome this problem, especially when comparing uninfected to infected plants and also between two infected plants of the same host species. Differences in plant size between replications were accounted for by

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considering replication as a blocking variable and analyzing each experiment as a RCBD. In addition, the MIXED model approach also enabled assignment of replication and cage nested within each replication as random factors. Furthermore, previous work supported use of settling behavior as an indicator of feeding and reproduction. For example, as demonstrated in chapter II, starved psyllids move to and begin settling on plants immediately upon release. Further, it was demonstrated that this behavior is likely accompanied by feeding, given that psyllids (both female and male) that are denied a food source do not live longer than 72h, and most die much sooner. Also, in this previous work it was shown that *B. cockerelli* females laid their eggs wherever they settled (chapter II) probably because the cues that indicate host nutritional quality for the female can also stimulate oviposition (Mitchell 1981). Thus, settling is likely to be a good indicator of feeding and subsequent oviposition in *B. cockerelli* and is the next best alternative to EPG studies that directly measure feeding.

This study clearly indicated that *B. cockerelli* adults preferentially settled on uninfected potato plants and other Lso-uninfected hosts compared to Lso infected hosts (Fig A.20 –A.27). In case of tomato three weeks after Lso-infection preference was exhibited by Lso-free psyllids for Lso-infected tomato plant during initial hours of settling which could possibly indicate Lso-free psyllids to orient to Lso-infected plants for acquiring the pathogen. Several factors may guide psyllids in making a choice for a preferred host – if a host is infected or not and if so at what time the infection started, the disease type and degree of symptoms expressed by the plant at the time psyllids arrive, and the size of infected versus healthy plants. Although olfactory cues may guide the

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psyllids to locate their preferred host at a long range, results of this study suggest that psyllids may rely on visual cues, being more attracted to larger and healthy plants at least at a short range. Thus the role of visual and olfactory cues in the host selection behavior of *B. cockerelli* is emphasized. It is possible that plants emit certain volatiles common to all solanaceous hosts, but varying among species of this family. Furthermore, larger plants might logically be expected to have a greater capacity to produce these volatiles. However, this study did not attempt to quantify volatiles or distinguish between visual or olfactory cues.

Although it is unknown at this time if psyllids use visual and/or olfactory cues to detect diseased plants it appears that these cues vary with the length of time a plant has been infected. For example, psyllids settled uniformly on both infected and uninfected pepper plants (Fig A.24 and A.25). It is possible that the six week-old pepper plants were asymptomatic and, based on visual or olfactory cues, psyllids could not discriminate between the infected and uninfected plants. However, potato plants started to wilt at three weeks after Lso-infection and significantly more Lso-infected psyllids settled on the uninfected than infected hosts (Fig A.20h). Similarly, in the field, infected potato plants wilted and died rather quickly, in agreement with Buchman et al. (2012). It is possible that psyllids reject infected hosts based on visual or olfactory cues, or possibly after tasting. For example, it was shown by Mann et al. (2012) that citrus plants that lost their turgor were no longer preferred by Asian citrus psyllids (ACP), *D. citri.* ACP were initially attracted to '*Candidatus* Liberibacter asiaticus' (Las)-infected citrus plants by olfaction, presumably due to ACP upregulating methyl-salicylate as part of the

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plant defense system, and then were repelled to uninfected plants after initial tasting. Davis et al. (2012) reported similar results in the potato-Lso system. However, they made no mention of the health of Lso-infected potato plant. Thus, if volatiles from infected plants were attractive to psyllids then they should continue to feed. The findings of Davis et al. (2012) are epidemiologically important, but psyllids are not only guided by olfactory stimuli but also visual and gustatory stimuli, as reported by Zhao et al. (2013), Wenninger et al. (2009), and Sanchez (2008). Infection by Las causes citrus trees to develop yellow shoots and produce the bitter compounds limonin and nomilin (Zhao 2013). The color and volatile compounds emitted by young shoots may play an important role in guiding ACP to locate the host plants. However, no such symptoms of yellowing or production of bitter compounds have been reported on solanaceous hosts due to Lso-infection. Even a visual stimulus such as a yellow sticky card can be equally attractive to a psyllid in the presence or absence of olfactory stimuli (Taylor et al. 2014).

Majority of experiments reported here did not reveal any differences between Lso-infected and uninfected psyllids in their settling preference for Lso-infected or uninfected hosts and of the few that did, preference was exhibited for uninfected hosts with an exception on tomato where Lso-free psyllids initially oriented towards Lso-infected plants. It was hypothesized that cold psyllids would prefer to acquire the pathogen, perhaps to enhance their survival. In the studies on life history traits (chapter IV) we showed that Lso-infected immatures developed faster than the ones from Lso-free colony. However, survival of Lso-free psyllids was better than the psyllids that harbored the pathogen, in agreement with Nachappa et al. (2012). Furthermore, psyllids

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did not exhibit a settling preference for plants that had been infected longer and thus potentially had higher Lso titers and/or greater symptomology (e.g. plants infected six versus three weeks). It is intuitive to expect more volatiles would be emitted after more time has elapsed after inoculation and, thus, psyllid preference would be more pronounced. However, because no differences in settling behavior was observed between three and six week-old plants, this indicates settling behavior of psyllids may primarily be guided by visual cues. However, studies under dark conditions and/or olfactometer experiments are needed to test this hypothesis.

Hypothesis testing of SLN being a possible reservoir host in the LRGV of Texas was carried out using Lso-acquisition studies in the laboratory and Lso-retention studies in the field. Laboratory results indicate that *B. cockerelli* was able to acquire Lso from Lso-infected SLN plants and transmit it back to healthy potato. However, it is imperative to determine if Lso-infected SLN plants are able to retain Lso through the heat of summer in the LRGV of Texas. Both the psyllid and Lso have a preferred optimum temperature of 27˚C (Munyaneza 2010) and are sensitive to high temperatures (Pletch 1947) beyond 35˚C (Munyaneza et al. 2012). *B. cockerelli* migrates northwards (Pletch 1947, Wallis 1955), disappearing from the LRGV and south Texas when temperatures start exceeding physiological tolerances of psyllids. It is not clear if Lso-infected wild perennial solanaceous hosts such as SLN are able to retain Lso until psyllids reappear in the LRGV the following winter. Samples of leaves and shoots were collected from Lso-infected SLN plants during September 2013 (SLN was planted in January 2013 and had been outside in the field throughout the summer of 2013) revealing that Lso was absent

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in these previously infected plants. Previous reports suggested that PCR diagnostics of '*Ca*. Liberibacter' species may be more reliable in lateral stolons and/or plant roots; testing of lateral stolons and roots collected at the same time is currently underway. The possibility of SLN serving as a reservoir host in the LRGV is questionable if they do not harbor Lso in the roots or stolons. SLN is a stolon producing plant and its translocation pattern could be similar to potato, making detection of Lso relatively difficult compared to tomato, eggplant and pepper. Further acquisition studies using Lso-free psyllids will confirm whether Lso is present in SLN plants at titers too low for PCR detection, but high enough to be acquired by the insects.

Conclusions. Contrary to our hypothesis and reports in the literature, psyllids invariably preferred uninfected plants of all hosts, except with three week-old Lso-infected tomato plants under laboratory conditions. Field results demonstrated that resident psyllids preferred to settle on five week-old uninfected potato plants compared to infected plants. Together the laboratory and field results suggest that psyllids may be predominantly guided to their hosts by a visual stimulus at least at a short range, as they did not prefer infected plants. It is certain that plants infected with Lso emit certain volatiles but, in the present study, it was clearly evident that these volatile cues were either too weak for attraction or else psyllids were choosing healthy and robust plants, indicating visual cues to be more important than olfactory stimuli in the host selection process. The disagreement between published reports and the present study suggests that several factors may be important in host selection by potato psyllids. Y-tube olfactometer studies are underway to test preferences by psyllids for Lso-infected or

uninfected hosts. These studies should help to elucidate important differences between olfactory and visual responses. The possibility of SLN serving as a reservoir host for Lso in the LRGV of Texas is still under investigation. Although psyllids were able to acquire Lso from infected SLN plants and transmit it to potato, retention of Lso by infected SLN plants under natural conditions is vital to determine its potential as a reservoir host. Above ground portions of SLN tested negative for Lso, but below ground stolons and roots will need additional testing. Insect acquisition studies are needed to confirm results.

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CHAPTER VI

CONCLUSIONS

SUMMARY

Research was conducted on host preferences, adaptation, and behavior of the potato psyllid, *B. cockerelli*, and transmission of the bacterial pathogen, '*Candidatus* Liberibacter solanacearum' among wild and cultivated solanaceous hosts in the Lower Rio Grande Valley of Texas. Results are summarized as conclusions and recommendations for future use and presented herein.

Preferential host responses were exhibited by *B. cockerelli* in the field study reported here. Field studies on settling behavior of *B. cockerelli* revealed that psyllid adults preferred potato based on significantly higher numbers that settled on pairs that contained potato. Next to potato tomato was most preferred but, was not significantly different from potato. When potato started to senesce, psyllids dispersed to other hosts, i.e. tomato, pepper, eggplant and silverleaf nightshade (SLN). Plant size strongly influenced psyllid adult preference and settling. Based on host plant densities, single plant plots are equally attractive to adults as larger plot densities, and even solitary plants could be good indicators of psyllid activity. An ideal time to sample psyllids in field on the plants is around 12pm when psyllids are settled on the plants. Under field conditions psyllids are more active morning and afternoon and their abundance stabilizes around 12pm.

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Together, the field and laboratory observations suggest that *B. cockerelli* preferred cultivated hosts over the wild host. Settling behavior of adults is a good indicator of feeding and oviposition preference given that starvation quickly leads to mortality, and that the mother and her offspring share the same feeding niche. In the laboratory, psyllids preferred eggplant, potato and pepper equally but preference was significantly different from tomato and SLN. SLN was the least preferred host among all five hosts. Pre-adaptation experiments revealed that, irrespective of the host plant on which the psyllids were raised, eggplant was strongly preferred over tomato in the laboratory. Furthermore, when small and large host plants were offered to psyllids to choose from, they demonstrated a strong preference for the larger of the two hosts, irrespective of the host plants tested. It is likely that psyllids combine both olfactory and visual stimuli to locate their host from a long range, and at a short range may rely on tactile and gustatory stimuli to choose its preferred host.

The performance of the potato psyllid was studied on SLN, the wild solanaceous host of the potato psyllid and Lso. Investigations elucidated significant differences in life history and life table parameters of both Lso-infected and uninfected colonies of the potato psyllid on SLN in comparison with its main host, potato. It was found that Lso-infectivity influenced overall nymphal survivorship and Lso-free colony individuals survived better than the Lso-infected ones on both hosts (potato and SLN). However, Lso-infected potato psyllid nymphs had a shorter developmental time (i.e. they developed faster compared to cold colony nymphs on both potato and SLN). Females from Lso-free colony lived longer on potato compared to SLN, and vice versa, indicating

an interaction effect between host and pathogen. Life table parameters (i.e., intrinsic rate of increase, net reproductive rate, mean generation time, doubling time, and final rate of natural increase) showed that growth and survival of both Lso-infected and Lso-free potato psyllids were highest on potato and these were significantly different on SLN indicating that SLN is an inferior host to potato. Furthermore, influence of host was more pronounced than Lso infection on immature survival than on female performance.

Contrary to the hypothesis of this study and published literature, psyllids preferred uninfected hosts of all five species tested and, in most cases, did not exhibit any preference at all for Lso-infected or uninfected potato, tomato or pepper. Only with three week-old tomato plants was there a preference exhibited by Lso-free psyllids for the Lso-infected tomato. It could possibly be that Lso-free psyllids tend to move to Lso-infected hosts to acquire the pathogen. Results from field studies demonstrated that significantly more resident psyllids settled on uninfected potato plants than plants that were infected with Lso when five weeks-old. Although previous results demonstrate the importance of olfactory cues to guide host orientation of psyllids, the results herein demonstrate that psyllids were more likely led by visual cues as they preferred plants that were healthy and vigorous as opposed to sick and dying plants. Taylor et al. (2014) emphasized the importance of visual cues in guiding *B. cockerelli* to large yellow sticky traps in potato fields. In comparisons where diseased plants were not symptomatic, the psyllids did not exhibit a choice, but instead settled equally on both hosts. Thus it was clearly evident that either volatile cues from Lso-infected plants were not strong enough for attraction, or else they were repelling the adults. Other factors in addition to volatiles

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may be responsible for attracting psyllids to host plants. Further studies using an olfactometer would help elucidate some of these factors. It was further determined that psyllids are capable of transmitting Lso from potato to SLN and vice versa. Psyllids tested positive for Lso within two weeks of feeding on Lso-infected SLN plants. The potential for Lso to be carried over through the summer in SLN needs more study.

The experiments reported here have illuminated settling response behaviors; however, further investigations are needed to elucidate the mechanisms that lead psyllids to make host choices. It is possible that volatiles specific to solanaceous plants may serve as an initial attractant at a distance, and further visual, tactile or gustatory stimuli may serve as a short range cue that determines the acceptability of the plant species for settling and subsequent feeding and oviposition.

Although substantial research efforts are being diverted towards management of the potato psyllid and ZC, both continue to remain a serious threat to commercial potato production. Though primarily a pest of the solanaceous plants, the potato psyllid attacks and transmits Lso to these hosts. However, alternate hosts are very important considerations in the epidemiology and management of insect vectors, as they enable survival of pest and pathogen in the absence of favored or cultivated hosts. Understanding host plant distribution patterns can provide useful information on pathogen spread and transmission by their vectors. Work with vector-borne diseases, mechanisms of pathogen transmission play a key role in the disease cycle. The transmission process is initiated via Lso-infected psyllids or psyllids acquiring Lso from diseased hosts, in which case both the infected psyllid and the infected host play a

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significant role in the spread of ZC disease. Given the importance of alternate host plant epidemiology in the management of the potato psyllid and ZC, a fundamental understanding of psyllid biology and population dynamics in relation to its host plants is important to support applied research.

Being a migratory and a seasonal pest, causing damage both directly as a pest and indirectly as a plant pathogen vector, sampling and monitoring of *B. cockerelli* requires continuous attention. Presently, insecticides play a pivotal role in the management of this important pest. A more rational approach would be to incorporate ecologically based principles that can work in tandem with insecticides to control the pest and the pathogen. For instance, the concept of trap cropping can be effectively used where insects are attracted to certain crops more than others. Because potato and tomato plants were preferred by psyllids more than the other solanaceous hosts that were tested for settling and oviposition, and tomato being preferred only next to potato, these findings could lead to further investigations on potato and tomato varieties for relative attraction to *B. cockerelli* populations migrating into the LRGV. Before planting the main crop, a border plot (early planting) of potato or tomato could be used to attract and kill the in-migrating adults. The border plants should be well established, however, compared to the main crop. The objective is to exploit the size effect (psyllids prefer larger hosts), with the trap crop planted as a border to the main crop (to make use of the edge effect). Thus, further spread into the interior field can be minimized.

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RECOMMENDATIONS

Attraction of *B. cockerelli* to certain hosts presents opportunities to evaluate preferred hosts as trap crops around potato fields. The concept of trap cropping can be used effectively whenever insects are attracted to certain hosts rather than the main crop, either as a food source, for oviposition, or both. Accordingly, a border of potato or tomato planted well before the main crop could serve as a trap crop to attract migrating adults and thus detection and management strategies using insecticides could be concentrated in a smaller area. *B. cockerelli* adults were most likely to be observed on plants at mid-day and exhibit greater movement during mornings and late afternoons. Thus, adults should ideally be sampled on plants during late morning to early afternoon to maximize sampling efforts.

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REFERENCES

Abdullah, N. M. M. 2008. Life history of the potato psyllid *Bactericera cockerelli* (Hompotera: Psyllidae) in controlled environmental agriculture in Arizona. Afr J. Agric. Res. 3: 60-67.

Al-Jabr, A. M. 1999. Integrated pest management of tomato/ potato psyllid, *Bactericera cockerelli* (Sulc) (Homoptera: Psyllidae) with emphasis on its importance in greenhouse grown tomatoes. Ph.D. Dissertation. Col. State Univ. Fort Collins.

Alvarado, V. Y., O. Duncan, D. Odokonyero, and H. B. Scholtoff. 2010. Quantifying '*Candidatus* Liberibacter' and other microbes in psyllids in relation to insect colony behavior and ZC disease. *In* F. Workneh, and C. M. Rush [eds]. Proc. 10th Annual Zebra Chip Rep. Session. Dallas, TX. Pp.24-28.

Anderson, P., C. Lofstedt, and P. A. Hamback. 2013. How insects sense olfactory patches – the spatial scaling of olfactory information. Oikos 122: 1009-1016.

Ballabeni, P., M. Wlodarczyk, and M. Rahier. 2001. Does enemy-free space for eggs contribute to a leaf beetle's oviposition preference for a nutritionally inferior host plant? Funct. Ecol. 15: 318-324.

Berdegene, M., S. R. Reitz, and J. T. Trumble. 1998. Host plant selection and development in *Spodoptera exigua*: Do mother and offspring know best? Entomol. Exp. Appl. 89: 57-64.

Bernays, E. A., R. F. and Chapman. 1977. Deterrent chemical as a basis of oligophagy in *Locusta migratoria* (L.). Ecol. Entomol. 2: 1-18.

Binkley, A. M. 1929. Transmission studies with the new psyllid-yellows disease of solanaceous plants. Science 70: 615.

Birch, L. C. 1948. The intrinsic rate of natural increase of an insect population. J. Anim. Ecol. 17: 15-26.

Bove, J. M. 2006. Huanglongbing: a destructive, newly-emerging, century-old disease of citrus. J. Plant Pathol. 88: 7-37.

Buchman, J. L., V. G. Sengoda, and J. E. Munyaneza. 2011a. Vector transmission efficiency of liberibacter by *Bactericera cockerelli* (Hemiptera: Triozidae) in zebra chip potato disease: Effects of psyllid life stage and inoculation access period. J. Econ. Entomol. 104: 1486-1495.

Buchman, J. L., B. E. Heilman, and J. E. Munyaneza. 2011b. Effects of liberibacter-infective *Bactericera cockerelli* (Hemiptera: Triozidae) density on zebra chip potato disease incidence, potato yield and tuber processing quality. J. Econ. Entomol. 104: 1783-1792.

Buchman, J. L., T. W. Fischer, V. G. Sengoda, and J. E. Munyaneza. 2012. Zebra chip progression: From inoculation of potato plants with liberibacter to development of disease symptoms in tubers. Am. J. Pot. Res. 89: 159-168.

Butler, C. D. and J. T. Trumble. 2010. Distribution and Phenology of the potato psyllid in southern California. *In* F. Workneh, and C. M. Rush [eds]. Proc. 10th Annual Zebra Chip Rep. Session. Dallas, TX. Pp.37-41.

Butler, C. D. and J. T. Trumble. 2012. The potato psyllid, *Bactericera cockerelli* (Sulc) (Hemiptera: Triozidae): life history, relationship to plant diseases, and management strategies. Terr. Arthropod Rev. 5: 87-111.

Butler, C. D., R. G. Novy, J. C. Miller, and J. T. Trumble. 2010. Alternative strategies: plant resistance and biological control. *In* F. Workneh, and C. M. Rush [eds]. Proc. 10th Annual Zebra Chip Rep. Session. Dallas, TX. Pp. 69-73.

Butler, C. D., B. Gonzalez, K. L. Manjunath, R. F. Lee, R. G. Novy, C. J. Miller, and J. T. Trumble. 2011. Behavioral responses of adult potato psyllid, *Bactericera cockerelli* (Hemiptera: Triozidae), to potato germplasm and transmission of *Candidatus* Liberibacter psyllaurous. Crop Prot. 30: 1233-1238.

Chapman, R. F. 1974. The chemical inhibition of feeding by phytophagous insects: A review. Bull. Entomol. Res. 64: 339-363.

Chapman, E. F., E. A. Bernays, and S. J. Simpson. 1981. Attraction and repulsion of the aphid, *Cavariella aegopodii*, by plant odors. J. Chem. Ecol. 14: 561-579.

Compere, H. 1916. Notes on the tomato psylla. Mon. Bull. Calif. St. Comm. Hortic..5: 189-191.

Cranshaw, W. S. 1993. An annotated bibliography of potato/tomato psyllid, *Paratrioza cockerelli* (Sulc) (Homoptera: Psyllidae). Colo. St. Ex. St. Bull. TB93-5.

Cranshaw, W. S. 1994. The potato (tomato) psyllid, *Paratrioza cockerelli* (Sulc), as a pest of potatoes. Pp. 83 – 95. *In* G. W. Zehnder, M. L. Powelson, R. K. Hansson, and K. V. Raman [eds.] Advances in potato pest biology and management. APA Press, St. Paul.

Crawford, D. L. 1914. A monograph of the jumping plant lice or psyllidae of the new world. U. S. Natl. Mus. Bul. 85: 186.

Cronin, J. T., and W. G. Abrahamson. 2001. Goldenrod stem galler preference and performance: Effects of multiple herbivores and plant genotypes. Oecologia 127: 87-96.

Crosslin, J. M., and G. Bester. 2009. First report of *Candidatus* Liberibacter psyllaurous in zebra chip symptomatic potatoes from California. Plant Dis. 93:551.

Crosslin, J. M., J. E. Munyaneza, J. K. Brown, and L. W. Liefting. 2010. Potato zebra chip disease: a phytopathological tale. Plant Health Progress. Online Publication. doi: 10.1094/PHP-2010-0317-1001-RV.

Crosslin, J. E., H. Lin, and J. E. Munyaneza. 2011. Detection of '*Candidatus* Liberibacter solanacearum' in the potato psyllid,, *Bactericera cockerelli* (Sulc), by conventional and real-time PCR. Southwest. Entomol. 36: 125-135.

Crosslin, J. M., P. B. Hamm, J. E. Munyaneza. 2012a. First report of zebra chip disease and '*Candidatus* Liberibacter solanacearum' on potatoes in Oregon and Washington State. Plant Dis. 96: 452.

Crosslin, J. M., N. Olsen, and P. Nolte. 2012b. First report of zebra chip disease and "*Candidatus* Liberibacter solanacearum" on potatoes in Idaho. Plant Dis. 96: 453.

Cunningham, J. P. 2012. Can mechanism help explain insect host choice? J. Evol. Biol. 25: 244-251.

Davis, A. C. 1937. Observations on the life history of *Paratrioza cockerelli* (Sulc) in southern California. J. Econ. Entomol. 30: 377-378.

Davis, T. S., D. R. Horton, J. E. Munyaneza, and P. J. Landolt. 2012. Experimental infection of plants with an herbivore-associated bacterial endosymbiont influences herbivore host selection behavior. PLoS ONE 7: 1-8.

Doyle, J. J., and J. L. Doyle. 1990. Isolation of plant DNA from fresh tissue. Focus 12: 13-15.

Drees, B. M. and J. Jackman. 1999. Field guide to Texas insects. Pp. 343. Gulf Publishing Company. Houston, TX.

Dwelle, R. B. 2003. Potato growth and development. Pp. 9-19. *In* J. C. Stark, and S. L. Love [eds.] Potato production systems. University of Idaho Agricultural Communications, Moscow, ID.

Essig. E. O. 1917. The tomato and laurel psyllids. J. Econ. Entomol. 10: 433-444.

Finch, S. **1978.** Volatile plant chemicals and their effect on host finding by the cabbage root fly (*Delia brassicae*). Entomol. Exp. Appl. 24: 150-159.

Fletcher, B. S., and R. J. Prokopy. 1991. Host location and oviposition in tephritid flies. Pp. 139-171. *In* W. J. Bailey, and J. Ridsdill-Smith [eds]. Reproductive behavior of insects-Individuals and Populations, Chapman and Hall, Melbourne.

Gao, F., J. Jifon, X. B. Yang, and T. X. Liu. 2009. Zebra chip disease incidence on potato is influenced by timing of potato psyllid infestation, but not by the host plants on which they are reared. Insect Sci. 16: 399-408.

Gill, G. 2006. Tomato psyllid detected in New Zealand. Biosecurity New Zealand 69: 10-11.

Goolsby, J. A., J. Adamczyk, B. Bextine, D. Lin, J. E. Munyaneza, and G. Bester. 2007. Development of an IPM program for management of the potato psyllid to reduce incidence of zebra chip disorder in potatoes. Subtrop. Plant Sci. 59: 85-94.

Goolsby. J. A., J. J. Adamczyk. J. M. Crosslin, N. N. Troxclair, J. R. Anciso, G. G. Bester, J. D. Bradshaw, E. D. Bynum, L. A. Carpio, D. C. Henne, A. Joshi, J. E. Munyaneza, P. Porter, P. E. Sloderbeck, J. R. Supak, C. M. Rush, F. J. Willet, B. J. Zechmann, and B. A. Zens. 2011. Regional monitoring of psyllid populations and the associated pathogen, *Ca.*Liberibacter psyllasurous. *In* F. Workneh, A. Rashed, and C. M. Rush [eds]. Proc. 11th Annual Zebra Chip Rep. Session. San Antonio, TX. Pp.12-16.

Hail, D. A., S. Dowd, W. H. Hunter, and B. Bextime. 2010. Investigating the transcriptome of the potato psyllid (*Bactericera cockerelli*): Toward an RNAi based management strategy. *In* F. Workneh, and C. M. Rush [eds]. Proc. 10th Annual Zebra Chip Rep. Session. Dallas, TX. Pp.183-186.

Hamm, P. B., S. I. Rondon, J. M. Crosslin, and J. E. Munyaneza. 2011. A new threat in the Columbia basin of Oregan and Washington: Zebra chip. *In* F. Workneh, A. Rashed, and C. M. Rush [eds]. Proc. 11th Annual Zebra Chip Rep. Session, San Antonio, TX. Pp. 1-5.

Hansen, A. K., J. T. Trumble, R. Stouthamer, and T. D. Paine. 2008. A new Huanglongbing (HLB) species 'Candidatus Liberibacter psyllaurous', found to infect tomato and potato, is vectored by the psyllid *Bactericera cockerelli* (Sulc). Appl. Env. Microbiol. 74: 5862-5865.

Haysom, K. A., and J. C. Coulson. 1998. The Lepidoptera fauna associated with *Calluna vulgaris*: Effects of plant architecture on abundance and diversity. Ecol. Entomol. 23: 377-385.

Hendrichs, J., B. L. Kaysoyannos, D. R. Papaj, and R. J. Prokopy. 1991. Sex differences in movement between natural feeding sites and tradeoffs between food consumption, mating success and predator evasion in Mediterranean fruit flies (Diptera: Tephritidae). Oecologia 86: 223-231.

Henne, D. C. 2012. Potato psyllid insecticide evaluation trials in the Lower Rio Grande Valley of Texas. *In* F. Workneh, A. Rashed, and C. M. Rush [eds.]. Proc. 12th Annual Zebra Chip Rep. Session. San Antonio, TX. Pp.135-139.

Henne, D. C., L. Paetzold, F. Workneh, and C. M. Rush. 2010a. Evaluation of potato psyllid cold tolerance, overwintering survival, sticky trap sampling, and effects of liberibacter on potato psyllid alternate host plants. *In* F. Workneh, and C. M. Rush [eds]. Proc. 10th Annual Zebra Chip Rep. Session. Dallas, TX. Pp.149-153.

Henne, D.C., F. Workneh, A. Wen, J. A. Price, J. S. Pasche, N. C. Gudmestad and C. M. Rush. 2010b. Characterization and epidemiological significance of potato plants grown from seed tubers affected by zebra chip disease. Plant Dis. 94: 659 – 665.

Henne, D. C., F. Workneh, and C. M. Rush. 2010c. Movement of *Bactericera cockerelli* (Heteroptera: Psyllidae) in relation to potato canopy structure, and effects on potato tuber weights. J. Econ. Entomol. 103: 1524-1530.

Henne, D. C., J. A. Goolsby, T. E. Mirkov, and J. E. Munyaneza. 2011. Potato psyllid and zebra chip disease management in South Texas. *In* F. Workneh, A. Rashed, and C. M. Rush [eds].Proc. 11th Annual Zebra Chip Rep. Session, San Antonio, TX. Pp.41-45.

Henne, D. C., F. Workneh and C. M. Rush. 2012a. Spatial patterns and spread of potato zebra chip disease in the Texas Panhandle. Plant Dis. 96: 948-956.

Henne, D., J. Anciso, J. Bradshaw, S. Whipple, L. Carpio, G. Schuster, J. Richmond, S. Walker, P. Porter, S. Barrett, J. Willett, J. M. Mathews, K. Deroo, P. Seger, J. Crosslin, L. Hamlin, and J. Goolsby. 2012b. Overview of the 2011-2012 potato psyllid areawide monitoring program. *In* F. Workneh, A. Rashed, and C.M. Rush [eds]. Proc. of the 12th Annual Zebra Chip Rep. Session. San Antonio, TX. Pp.1-4.

Henne, D., J. Bradshaw, S. Whipple, L. Carpio, G. Schuster, P. Porter, S. Barrett, J. Willett, J. M. Mathews, K. Deroo, P. Seger, J. Crosslin, and L. Hamlin. 2013. Overview of the 2012-2013 potato psyllid areawide monitoring program. Proc. 13th Annual Zebra Chip Rep. Session, San Antonio TX (in press).

Hokkanen, H. 1991. Trap cropping in pest management. Annu. Rev. Entomol. 36: 119-138.

Hsiao, H. T. 1969. Chemical basis of host selection and plant resistance in oligophagous insects. Entomol. Exp. Appl. 12: 777-788.

Huang, Y. B., and H. Chi. 2012. Assessing the application of the jackknife and bootstrap techniques to the estimation of the variability of the net reproductive rate and gross reproductive rate: A case study in *Bactrocera cucurbitae* (Coquillett) (Diptera: Tephritidae). J. Agri. Fore. 61: 37-45.

Jackson, B. C., J. Goolsby, A. Wyzykowski, N. Vitovsky, B. Bextine. 2009. Analysis of genetic relationships between potato psyllid (*Bactericera cockerelli)* populations in the United States, Mexico and Gautemala using ITS2 and Inter Simple Sequence Repeat (ISSR) data. Subtrop. Plant Sci. 61: 1-5.

Janes, M. J. 1939. Observations on the potato psyllid in southwest Texas. J. Econ. Entomol. 32: 468.

Keyfitz, N. 1977. Introduction to the mathematics of populations. Addison-Wesley, Reading, MA.

Khan, Z. R., R. C. Saxena, and B. P. Rueda. 1988. Responses of rice infesting populations of *Nilaparvata lugens* (Homoptera:Delphacidae) to rice plants and Leersia grass and to their steam distillate extracts. J. Econ. Entomol. 81: 1080-1088.

Klyver, F. D. 1931. California psyllids of present and potential economic importance. Dept. Agric. Monthly Bull. State Calif. 20: 691-697.

Knowlton, G. E. 1933a. Aphid lion predators of the potato psyllid. J. Econ. Entomol. 26: 977.

Knowlton, G. E. 1933b. Ladybird beetles as predators of the potato psyllid. Can. Entomol. 65: 241-243.

Knowlton, G. F., and M. J. Janes 1931. Studies on the biology of *Paratrioza cockerelli* (Sulc). Ann. Entomol. Soc. Am. 24: 283-290.

Knowlton, G. F., and W. L. Thomas. 1934. Host plants of the potato psyllid. J. Econ. Entomol. 27: 547.

Lacey, L. A., F. de la Roza, and D. R. Horton. 2009. Insecticidal activity of entomopathogenic fungi (Hypocreales) for potato psyllid, *Bactericera cockerelli* (Hemiptera: Triozidae): development of bioassay techniques, effect of fungal species and stage of the psyllid. Biocont. Sci. Tech 9: 957-970

Lacey, L. A., T. –X. Liu, J. L.Buchman, J. E. Munyaneza, J. A. Goolsby, and D. R.

Horton. 2011. Entomopathogenic fungi (Hypocreales) for control of potato psyllid, *Bactericera cockerelli* (Sulc) (Hemiptera: Triozidae) in an area endemic for zebra chip disease of potato. Biol. Cont. 56: 271-278.

Lawton. J. H. 1978. Host plant influences on insect diversity: The effects of space and time. Symp. R. Entomol. Soc. Lond. 9: 105-125.

Lawton, J. H. 1983. Plant architecture and the diversity of phytophagous arthropods. Annu. Rev. Entomol. 28: 23-39.

Lehman, R. S. 1930. Some observations on the life history of the tomato psyllid, (*Paratrioza cockerelli* Sulc) (Homoptera). J. New York Entomol. S. 38: 307-312.

Levy J., E. Pierson, R. Aravind, D. C. Gross and C. Tamborindeguy. 2011. Translocation of *Candidatus* Liberibacter s*olanacearum,* the zebra chip pathogen in potato and tomato. Phytopathology 101: 1285-1291.

Liefting, L. W., Z. C. Perez-Egusquiza, G. R. G. Clover, and J. A. D. Anderson. 2008. A new '*Candidatus* Liberibacter' species in *Solanum tuberosum* in New Zealand. Plant Dis. 92: 1474.

Liefting, L. W., P. W. Sutherland, L. I. Ward, K. L. Paice, B. S. Weir, and R. G. Clover. 2009a. A new '*Candidatus* Liberibacter' species associated with diseases of solanaceous crops. Plant Dis. 93:208-214.

Liefting, L. W., B. S. Weir, S. R. Pennycook, and G. R. G. Clover. 2009b. *'Candidatus* Liberibacter solanacearum', associated with plants in the family Solanaceae. Int. J. Sys. Evol. Microbiol. 59: 2274-2276.

List. G.M. 1939. The effect of temperature upon egg deposition, egg hatch and nymphal development of *Paratrioza cockerelli* (Sulc). J. Econ. Entomol. 32: 30-36.

List, G. M. and L. B. Daniels. 1934. A promising control for potato yellows of potatoes. Science 79: 79.

Liu, D. and J. T. Trumble. 2004. Tomato psyllid behavioral responses to tomato plant lines and interactions of plant lines with insecticides. J. Econ. Entomol. 97: 1078-1085.

Liu, D., **and J. T. Trumble. 2006.** Ovipositional preferences, damage thresholds, and detection of the tomato-potato psyllid *Bactericera cockerelli* (Sulc) (Homoptera: Psyllidae) on selected tomato accessions. Bull. Entomol. Res. 96: 197-204.

Liu, D., and J. T. Trumble. 2007. Comparative fitness of invasive and native populations of the potato psyllid (*Bactericera cockerelli*). Entomol. Exp. Appl. 123: 35-42.

Liu, D., L. Johnson, J. T. Trumble. 2006a. Differential responses to feeding by the tomato/potato psyllid between two tomato cultivars and their implications in establishment of injury levels and potential of damaged plant recovery. Insect Sci. 13: 195-204.

Liu, D., **J. T. Trumble, and R. Stouthamer. 2006b.** Genetic differentiation between eastern populations and recent introductions of potato psyllid (*Bactericera cockerelli*) into western North America. Entomol. Exp. Appl. 118: 177-183.

Liu, G., and R. M. Wilkins. 1988. Mechanisms of rice varietal resistance to the whitebacked planthopper, *Sogatella furcifera.* Brighton Crop Prot. Conf. – Pests Dis. 3: 1227-1232.

Maia, A. H. N., A. J. B. Luiz, and C. Campanhola. 2000. Statistical inference on associated fertility life table parameters using jackknife technique.: Computational aspects. J. Econ. Entomol. 93: 511-518.

Mann, R. S., J. G. Ali, S. L. Hermann, S. Tiwari, K. S. Pelz-Stelinski, H. T. Alborn, L.T. Stelinski. 2012. Induced release of a plant-defense volatile 'deceptively' attracts insect vectors to plants infected with a bacterial pathogen. PLoS ONE 8: 1-13.

Martini, X., S. Seibert, S. M. Prager, and C. Nansen. 2012. Sampling and interpretation of psyllid nymph counts in potatoes. Entomol. Exp. Appl. 1-8.

Mayhew, P. J. 1997. Adaptive patterns of host-plant selection by phytophagous insects. Oikos 79: 417-428.

Mayhew, P. J. 2001. Herbivore host choice and optimal bad motherhood. Trends Ecol. Evol. 16: 165-167.

Mcindodo, N. E. 1926. An insect olfactometer. J. Econ Entomol. 19: 545-571.

Miller, J. R., and K. Strickler. 1984. Plant-herbivore relationships, finding and accepting host plants. Pp. 127-137. *In* W. J. Bell and R. T. Carde [eds.]. Chemical ecology of insects. Chapman & Hall, London.

Miller, J. C., D. C. Scheuring, J. Koym, S. D. Turner, R. G. Novy. J. T. Trumble, C. D. Butler, C. Nansen, K. Vaughn, T. X. Liu, J. E. Munyaneza, E. Pierson, C. M. Rush, J. Jifon, R. French, C. Tamborindeguy, and P. Porter. 2010. Progress in identifying host plant tolerance/ resistance to ZC in potato

germplasm. *In* F. Workneh, and C. M. Rush [eds]. Proc. 10th Annual Zebra Chip Rep. Session. Dallas, TX, Pp.96-98.

Mitchell, R. 1981. Insect behavior, resource and exploitation and fitness. Annu. Rev. Entomol. 26: 373-96.

Munyaneza, J. E. 2010. Psyllids as vectors of emerging bacterial diseases of annual crops. Southwest. Entomol. 35: 417-477.

Munyaneza, J. E. 2012. Zebra chip disease of potato: Biology, Epidemiology, and Management. Am. J. Potato Res. 89: 329-350.

Munyaneza, J. E., J. M. Crosslin, and J. E. Upton, J. E. 2007a. Association of *Batericera cockerelli* (Homoptera: Psyllidae) with "zebra chip", a new potato disease in southwestern United States and Mexico. J. Econ. Entomol. 100: 656-663.

Munyaneza, J. E., J. A. Goolsby, J. M. Crosslin, and J. E. Upton. 2007b. Further evidence that zebra chip potato disease in the lower Rio Grande Valley of Texas is associated with *Bactericera cockerelli*. Subtrop. Plant Sci. 59:30-39.

Munyaneza, J. E., J. L. Buchman, J.E. Upton, J.A. Goolsby, J. M. Crosslin, G. Bester, G. P. Miles, and G. S. Venkatesan. 2008. Impact of different potato psyllid populations on zebra chip disease incidence, severity, and potato yield. Subtrop. Plant Sci. 60: 27-37.

Munyaneza, J. E., J. L. Buchman, J. A. Goolsby, A. P. Ochoa, and G. Schuster. 2010a. Impact of potato planting timing on zebra chip incidence in Texas. *In* F. Workneh, and C. M. Rush [eds]. Proc. 10th Annual Zebra Chip Rep. Session. Dallas, TX. Pp. 106-109.

Munyaneza, J. E., J. L. Buchman, V. G. Sengoda, T. W. Fischer, G. Bester, R. Hoopes, C. Miller, R. G. Novy, P. Van Hest, and J. Nordgaard. 2010b. Potato variety screening trial for zebra chip resistance under controlled field cage conditions. *In* F. Workneh, and C. M. Rush [eds]. Proc. 10th Annual Zebra Chip Rep. Session. Dallas, TX. Pp. 200-203.

Munyaneza, J. E., J. L.Buchman, V. G. Sengoda, T. W. Fischer, and C. C. Pearson. 2011. Susceptibility of selected potato varieties to zebra chip potato disease. Am. J. Potato Res. 88: 435-440.

Munyaneza, J. E., V. G. Sengoda, J. L. Buchman, and T. W. Fischer. 2012. Effects of temperature on '*Candidatus* Liberibacter solanacearum' and zebra chip potato disease symptom development. Plant Dis. 96: 18-23.

Nachappa, P., J. Levy, E. Pierson, and C. Tamborindeguy. 2011. Diversity of endosymbionts in the potato psyllid, *Bactericera cockerelli* (Hemiptera:Triozidae), vector of zebra chip disease of potato. Curr. Microbiol. 62: 1510-1520.

Nachappa, P., A. A. Shapiro, and C. Tamborindeguy. 2012. Effect of '*Candidatus* Liberibacter solanacearum' on fitness of its insect vector, *Bactericera cockerelli* (Hempitera: Triozidae), on tomato. Phytopathology 102: 41-46.

Nachappa, P., J. Levy, E. Pierson, and C. Tamborindeguy. 2014. Correlation between "*Candidatus* Liberibacter solanacearum" infection levels and fecundity in its psyllid vector. J. Invetebr. Pathol. 115: 55-61.

Nansen, C., X. Martini, K. Vaughn, Y. Xue, C. Miller, and C.M. Rush. 2010. Non-insecticidal based approaches to psyllid management. *In* F. Workneh, and C. M. Rush [eds]. Proc. 10th Annual Zebra Chip Rep. Session. Dallas, TX. Pp 55-58.

Nanthagopal, R., **and S. Uthamasamy. 1989.** Life tables on the spotted bollworm, *E. vitella* (Fab.) on four species of cotton. Crop Protect. 8: 133-136.

Nelson, W. R**., T. W. Fischer, and J. E. Munyaneza. 2011.** Haplotypes of "*Candidatus* Liberibacter solanacearum" suggest long-standing separation. Eur. J. Plant Pathol. 130: 5-12.

Nolte, P., **N. Olsen, E. Wenninger, and M. Thornton. 2011.** Zebra chip found in Idaho. *In* F. Workneh, A. Rashed, and C. M. Rush [eds.]. Proc. 11th Annual Zebra Chip Rep. Session, San Antonio, TX. Pp. 6

Novy, R. G., J. Whitworth, J. Alvarez, J. T. Trumble, C. D. Butler, J. L. Buchman, and J. E. Munyaneza. 2012. Unique tri-species germplasm with multiple insect resistances and its use in breeding for resistance to psyllid/ZC. *In* F. Workneh, A. Rashed, and C. M. Rush [eds]. Proc. 12th Annual Zebra Chip Rep. Session. San Antonio, TX. Pp. 103-105.

Pack, H. J. 1930. Notes on miscellaneous insects of Utah. Utah. Agric. Exp. St. Bull. 216: 30.

Peng, L., J. T. Trumble, J. E. Munyaneza, and T. X. Liu. 2011. Repellency of a kaolin particle film to potato psyllid, *Bactericera cockerelli* (Hemiptera: Psyllidae) on tomato under laboratory and field conditions. Pest Mgt. Science 67:815-824.

Pierson, E., C. Miller, D. Scheuring, T. X. Liu, X. Yang, J. Jifon, D. Gross, R. Aravind, and J. Levy. 2010. Investigations on putative zebra chip (ZC) tolerant advanced selections. *In* F. Workneh, and C. M. Rush [eds]. Proc. 10th Annual

Zebra Chip Rep. Session. Dallas, TX Pp. 99-103.

Pletch, D. J. 1947. The potato psyllid *Paratrioza cockerelli* (Sulc), its biology and control. Mont. Agric. Exp. Stn. Bull. 446:95.

Prokopy, R. J., and Owens, E. D. 1983. Visual detection of plants by herbivorous insects. Annu. Rev. Entomol. 28: 337-364.

Raghu, S., A. I. D. Richard, and A. R. Clarke, A. R. 2004. Influence of host plant structure and microclimate on the abundance and behavior of a tephritid fly. J. Insect Behav. 17: 179–190.

Ravindran, A., J. Levy, E. Pierson, and D. Gross. 2011. Development of primers for improved detection of the potato zebra chip pathogen, '*Candidatus* Liberibacter solanacearum'. Plant Dis. 95: 1542-1546.

Richards, B. L. 1928. A new and destructive disease of the potato in Utah and its relation to the potato psylla. Phytopathology 18: 140-141.

Richards, B. L., and F. E. Stephens. 1932. Psyllid yellows in Utah. Pp: 10-13. *In:* Proceedings of the annual conference of the western plant quarantine Bd. Calif. Dep. Agric. Sp. Bull. 115.

Richards, B. L., and H. L. Blood. 1933. Psyllid yellows of the potato. J. Agr. Res. 46:189-216.

Robinson, A. F., C. C. Orr, and C. E. Heintz. 1978. Distribution of *Nothanguina phyllobia* and its potential as a biological control agent of silverleaf nightshade. J. Nematol. 10: 361-366.

Romney, V. E. 1939 Breeding areas of the tomato psyllid, *Paratrioza cockerelli* (Sulc). J. Econ. Entomol. 32: 150-151.

Rondon, S. A., Schreiber, A. Jensen, P. Hamm, J. E. Munyaneza, P. Nolte, N. Olsen, E. Wenninger, D. Henne, C. Wohleb, and T. Waters. 2012. Potato psyllid vector of zebra chip disease in the Pacific Northwest: Biology, ecology, and management. Oreg. Aes. Bull. 633, online: http://ir.library.oreganstate.edu/xmlui/bitstream/handle/1957/30058/pnw633.pdf.

Rowe, J. A. 1933. Potato Health Management. Pp. 112-113. ARS Press St. Paul. MN.

Rubio-Covarrubias, O. A., I. H. Almeyda-Leon, J. I. Moreno, J. A. Sanchez-Salas, R. F. Sosa, J. T. Borbon-Soto, C. D. Hernandez, J. A. Garzon-Tiznado, R. R. Rodriguez, and M. A. Cadena-Hinajosa. 2006. Distribution of potato purple

top and *Bactericera cockerelli* Sulc. in the main potato production zones in Mexico. Agric. Tec. Mexico. 32: 201-211.

Rush, C. M., D. C. Henne, F. Workneh, and L. Paetzold. 2010. Investigating titer variation of Candidatus Liberibacter solanacearum in individual psyllid populations. *In* F. Workneh, and C. M. Rush [eds]. Proc. 10th Annual Zebra Chip Rep. Session. Dallas, TX, Pp19-23.

Sanchez, A. 2008. Importance of visual stimuli and host plant odor in host finding by the Asian citrus psyllid *Diaphorina citri* Kuwayana (Hemiptera: Psyllidae) M. S. Thesis. Texas A&M University, Kingsville.

SAS Institute. 2011. SAS version 9.3. SAS Institute, Cary, NC.

SAS Institute. 2013. Enterprise Guide version 5.1. SAS Institute, Cary, NC.

Saxena, K. N. 1969. Patterns of insect-plant relationships determining susceptibility or resistance of different plants to an insect. Entomol. Exp. Appl. 12: 751-766.

Saxena, R. C. 1986. Biochemical bases of insect resistance in rice varieties. Pp. 142-149. *In* M. B. Green and P. Hedin [eds.]. Natural Resistance of Plants to Insects: Roles of Allelochemicals, American Chemical Society, Washington, DC.

Scheirs, J. and L. De Bruyn. 2002. Integrating optimal foraging and optimal oviposition theory in plant-insect research. Oikos 96: 187-191.

Secor, G. A., and V. V. Rivera-Varas. 2004. Emerging diseases of cultivated potato and their impact on Latin America. Rev. Latinoam. Papa (Supl.) 1: 1-8.

Secor, G. A., V. Revera-Varas, J. A. Abad, I. M. Lee, G. R. G. Clover, L. W. Liefting, X. Li, and S. H. De Boer. 2009. Association of '*Candidatus* Liberibacter solanacearum' with zebra chip disease of potato established by graft and psyllid transmission, electron microscopy, and PCR. Plant Dis. 93: 574-583.

Sengoda, G. S., J. E. Munyaneza, J. M. Crosslin, J. L. Buchman, and H. R. Pappu. 2010. Phenotypic and etiological differences between psyllid yellows and zebra chip diseases of potato. Am. J. Potato Res. 87: 41-49.

Sengoda, G. S. J. L. Buchman, D. C. Henne, H. R. Pappu, and J. E. Munyaneza. 2013. "*Candidatus* Liberibacter solanacearum" titer over time in *Bactericera cockerelli* (Hemiptera: Triozidae) after acquisition from infected potato and tomato plants. J. Econ. Entomol. 106: 1964-1972.

Stephens, D. W., and J. R. Krebs. 1996. Foraging theory. Princeton University Press, Princeton. NJ.

Swisher, K. D., J. E. Munyaneza, and J. M. Crosslin. 2012. High resolution melting analysis of the cytochrome oxidase I gene identifies three haplotypes of the potato psyllid in the United States. Environ. Entomol. 41: 1019-1028.

Taylor, N. M., R. C. Butler, J. Vereijssen, and M. M. Davidson. 2014. Trap color, size, and borders alter catches of *B. cockerelli* in a potato crop. Entomol. Exp. Appl. 150: 226-231.

Thinakaran, J., C. Tamborindeguy, E. Pierson, and D. Henne. 2012. Host selection by the potato psyllid, *Bactericera cockerelli* (Sulc) (Hemiptera: Triozidae). *In* F. Workneh, A. Rashed, and C. M. Rush [eds]. Proc. 12th Annual Zebra Chip Reporting Session. San Antonio, TX. Pp. 199-203.

Thinakaran, J., E. Pierson, M. Kunta, and D. Henne. 2013. Role of solanaceous alternative host in the transmission of the bacterial pathogen, '*Candidatus* Liberibacter solanacearum' (Lso) in the Lower Rio Grande Valley of Texas. Proc. 13th Annual Zebra Chip Rep. Session. San Antonio, TX. (in press).

Thomas, K. L., D. C. Jones, L. B. Kumarasinghe, J. E. Richmond, G. S. C. Gill, and M. S. Bullians. 2011. Investigations into the entry pathway for the tomato potato psyllid *Bactericera cockerelli*. NewZealand Plant Prot. 64: 259-268.

Thomson, R. L., J. T. Forsman, F. Sarda-Palomera, and M. Monkkonen. 2006. Fear factor: prey habitat selection and its consequences in a predation risk landscape. Ecography 29: 507-514.

Trevino, J., G. Schuster, S. D. Nelson, A. P. Ochoa, and J. E. Munyaneza. 2011. Effects of potato planting dates on psyllid populations and zebra chip incidence in Texas. Proc. 11th Annual Zebra Chip Rep. Session. Pp. 127-130.

Turlings, T. C., A. C. Davidson, and C. Tamo. 2004. A six-arm olfactometer permitting simultaneous observation of insect attraction and odor trapping. Physiol. Entomol. 29: 45-55.

Vargas-Madriz, H. N. Bautista-Martinez, J. Vera-Graziano, C. Garcia-Gutierrez, and C. Chavarin-Palacio. 2011. Life and fertility table of *Bactericera cockerelli* (Sulc) on two varieties of tomato in a greenhouse. Southwest. Entomol. 36: 413-422.

Wallis, R. L. 1946. Seasonal occurrence of the potato psyllid in the North Platte Valley. J. Econ. Entomol. 39:689-694.

Wallis, R. L. 1955. Ecological studies on the potato psyllid as a pest of potatoes. Tech. Bull. U.S. Dep. Agric. 1107: 1-24.

Walther, B. A., and A. G. Gosler. 2001. The effects of food availability and distance to protective cover on the winter foraging behavior of tits (Aves: *Parus*). Oecologia 129: 312-320.

Wen, A., I. Malik, V. Y. Alvarado, J. S. Pasche, X. Wang, W. Li, L. Levy, H. Lin, H. B. Scholthof, T. E. Mirkov, C.M. Rush, and N. C. Gudmestad. 2009. Detection, distribution, and genetic variability of '*Candidatus* Liberibacter' species associated with zebra complex disease of potato in North America. Plant Dis. 93:1102-1115.

Wenninger, E., **L. L. Stelinski, and D. G. Hall. 2009.** Role of olfactory cues, visual cues, and mating status in orientation of *Diaphorina citri* Kuwayana (Hemiptera: Psyllidae) to four different host plants. Environ. Entomol. 38: 225-234.

West, S. A., and J. P. Cunningham. 2002. A general model for host plant selection in phytophagous insects. J. Theor. Biol. 214: 499-513.

Willmer, P. G. 1982. Microclimate and the environmental physiology of insects. Adv. Insect Physiol. 16: 1-57.

Workneh, F., D. C. Henne, L. Paetzold, and C. M. Rush. 2011. Effect of temperature on potato psyllid reproduction and Liberibacter titer levels in tubers. Phytopathology 101: S194

Workneh, F., D. C. Henne, A. C. Childers, L. Paetzold, and C. M. Rush. 2012. Assessments of the edge effect in intensity of potato zebra chip disease. Plant Dis. 96: 943-947.

Wuriyanghan, H., C. Rosa, and B. W. Falk. 2011. Oral delivery of double-stranded RNAs and siRNAs induces RNAi effects in the potato tomato psyllid, *Bactericera cockerelli*. PLoS ONE 6: e27736. doi: 10.1371/journal.pone.0027736.

Yang, X. B., and T. X. Liu. 2009. Life history and life tables of *Bactericera cockerelli* (Hemiptera: Psyllidae) on egg plant and bell pepper. Environ. Entomol. 38: 1661-1667.

Yang, X. B., Y. M. Zhang, L. Hua, and T. X. Liu. 2010a. Life history and life tables of *Bactericera cockerelli* (Hemiptera: Psyllidae) on potato under laboratory and field conditions in the Lower Rio Grande Valley of Texas. J. Econ. Entomol. 103: 1729-1734.

Yang, X. B., Y. M. Zhang, L. Hua, L. N. Peng, J. E. Munyaneza, and T. X. Liu. 2010b. Repellency of selected biorational insecticides to potato psyllid, *Bactericera cockerelli* (Hemiptera: Psyllidae). Crop Prot. 29: 1329-1324.

Yang, X.B., Y. M. Zhang, D. C. Henne, and T.X. Liu. 2013. Life tables of *Bactericera cockerelli* (Hemiptera: Triozidae) on tomato under laboratory and field conditions in southern Texas. Fla. Entomol. 96: 904-913.

Zhao, J. P., H. T. Wang, X. N. Zeng, and P.P. Xue. 2013. Differences in selection behavior and chemical cues of adult asian citrus psyllids, *Diaphorina citri*, on healthy and huanglongbing infected young shoots of citrus plants. J. Agr. Sci. 5:83:91.

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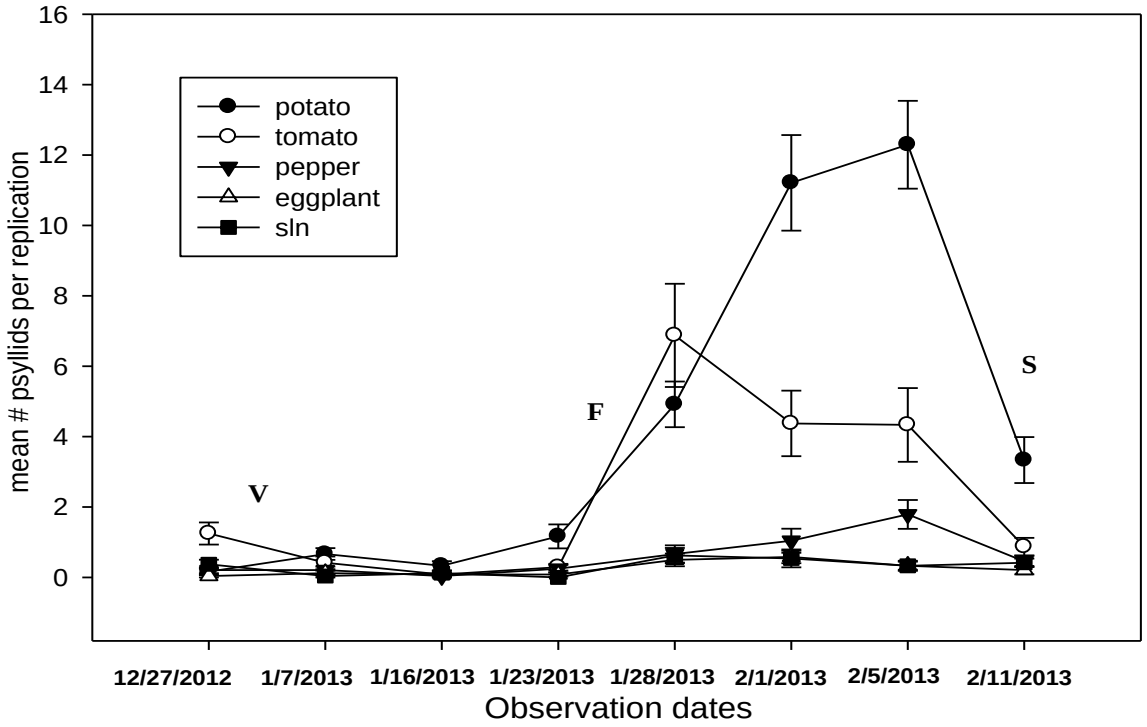
APPENDIX A

FIGURES AND TABLES

Figure 1 Treatment combinations used in field and laboratory experiments

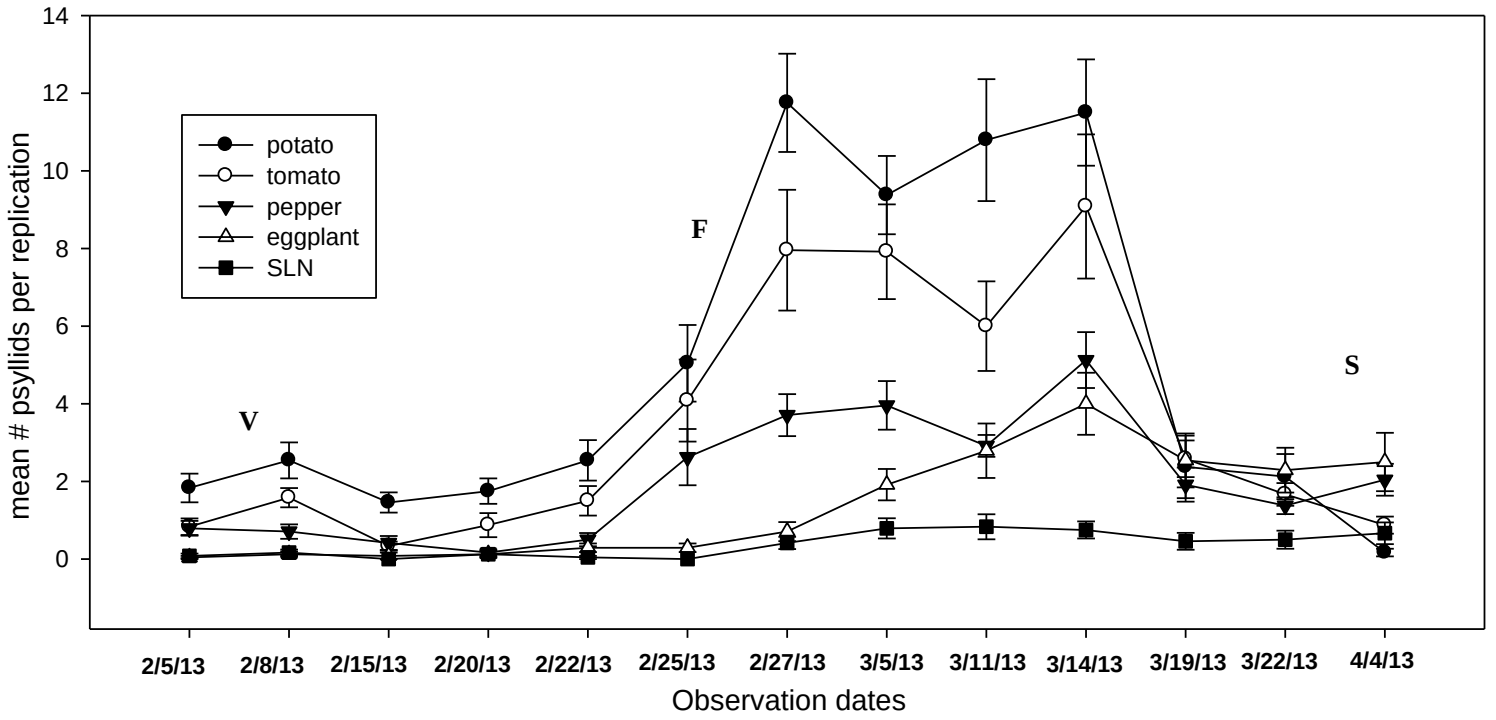
PP	PT	PC	PE	PS	|	
TT	TC	TE	TS		|	
CC	CE	CS				1 replication
EE	ES				|	
SS					|	

Figure 2 Field settling of *B. cockerelli* adults (host-wise comparison) Trial 1



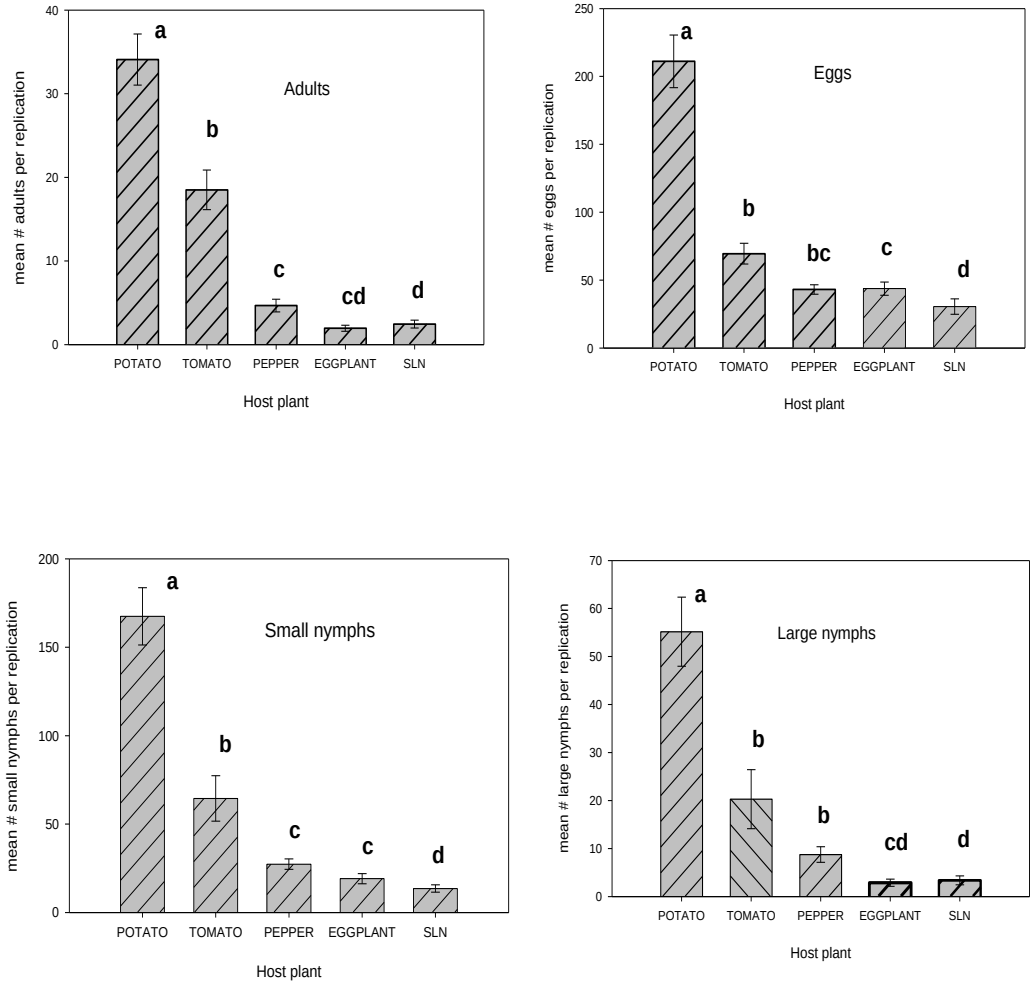
Number of *B. cockerelli* adults that settled on the respective host plants. Different phenological stages are represented by V- vegetative stage, F-flowering (except SLN), S- senescence (only potato)

Figure 3 Field settling of *B. cockerelli* adults (host-wise comparison) Trial 2



Number of *B. cockerelli* adults that settled on the respective host plants. Different phenological stages are represented by V- vegetative stage, F-flowering (except SLN), S- senescence (only potato)

Figure 4 Number of *B. cockerelli* adults, eggs, small and large nymphs – Field trial 1



Data pooled across three time points for eggs and nymphs and eight time points for adults. Bars represent mean numbers per replication. Error bars show ±SE. Bars denoted by the same letter are not significantly different from each other at P=0.05 level.

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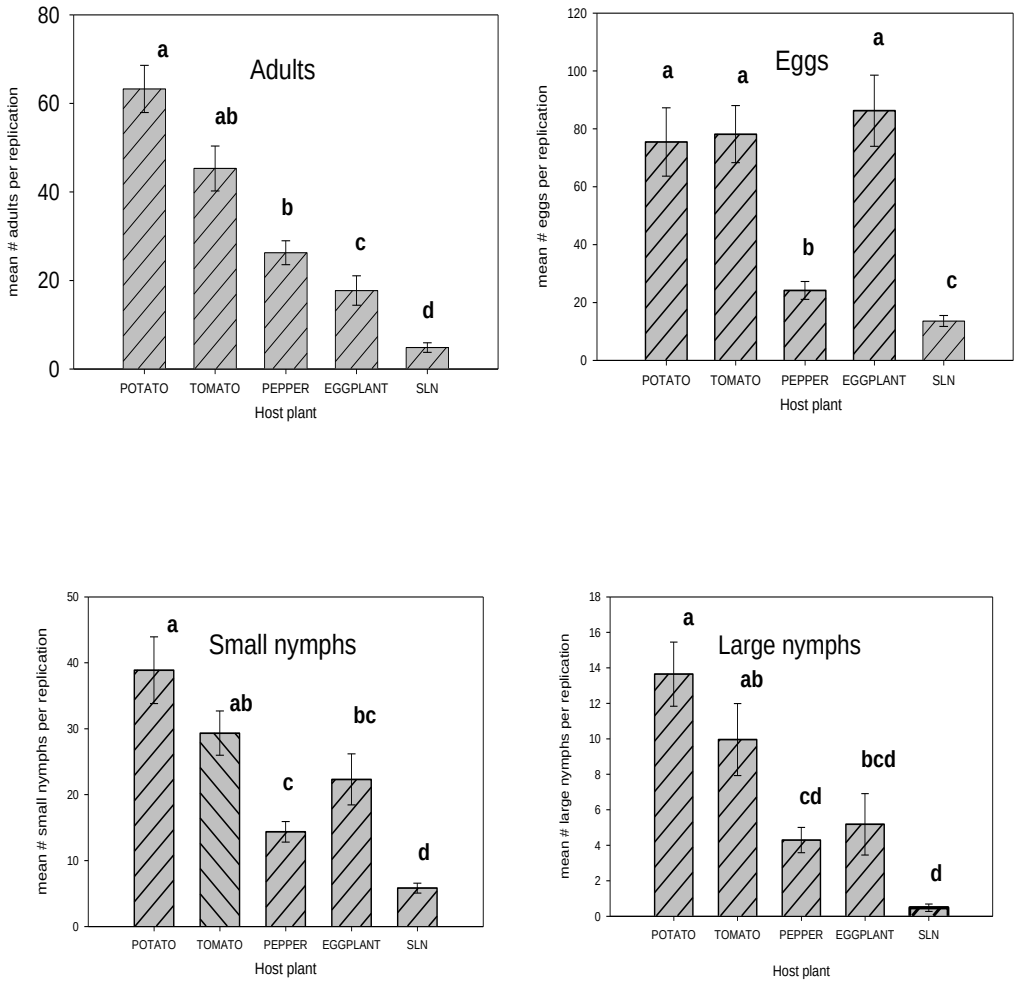
Figure 5 Number of *B. cockerelli* adults, eggs, small and large nymphs – Field Trial 2



Data pooled across two time points for eggs and nymphs and 13 time points for adults. Bars represent mean numbers per replication. Error bars show ±SE. Bars denoted by the same letter are not significantly different from each other at P=0.05 level.

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Figure 6 Potato-tomato host pair comparison at different stages of plant growth (growth stages are mentioned with reference to growth of potato)

a. At emergence

b. Early growth

c. Vegetative Phase

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d. Tuber formation

e. Senescence

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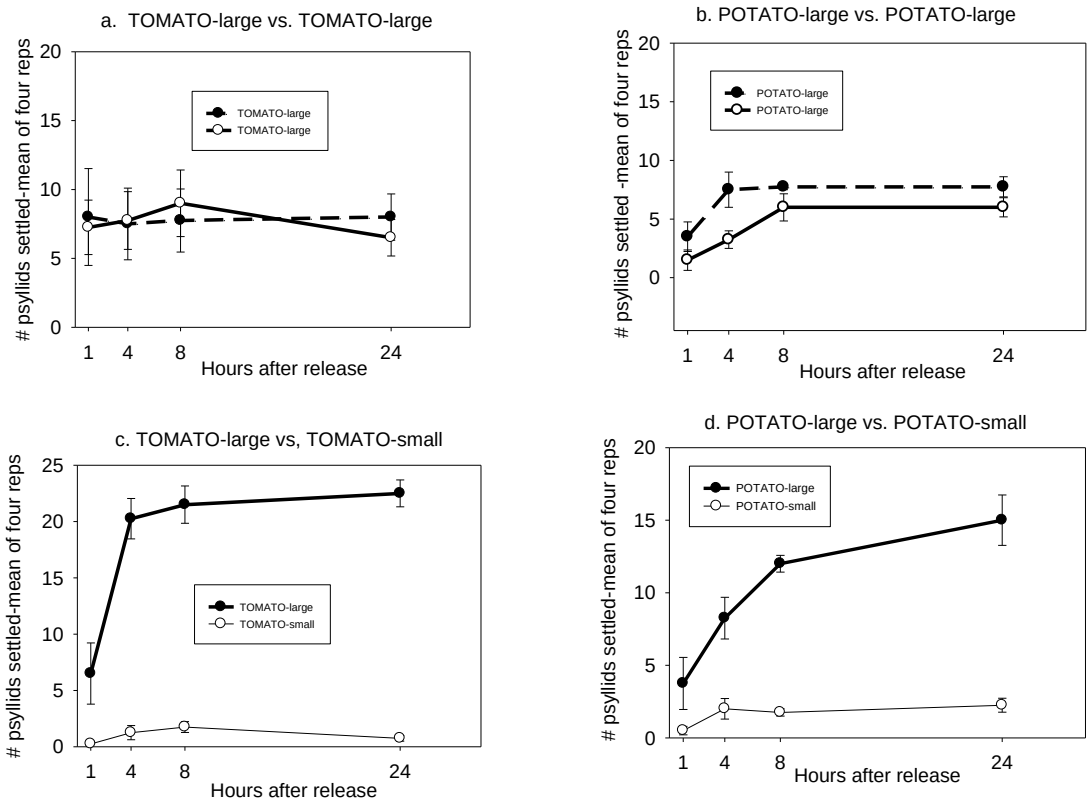
Figure 7 Plant size same host comparison- (large vs. large, large vs. small)



Graphs illustrate adult psyllid settling on the individual hosts in each pair over a 24h period. Observations were recorded at 1, 4, 8 and 24h after initial psyllid release.

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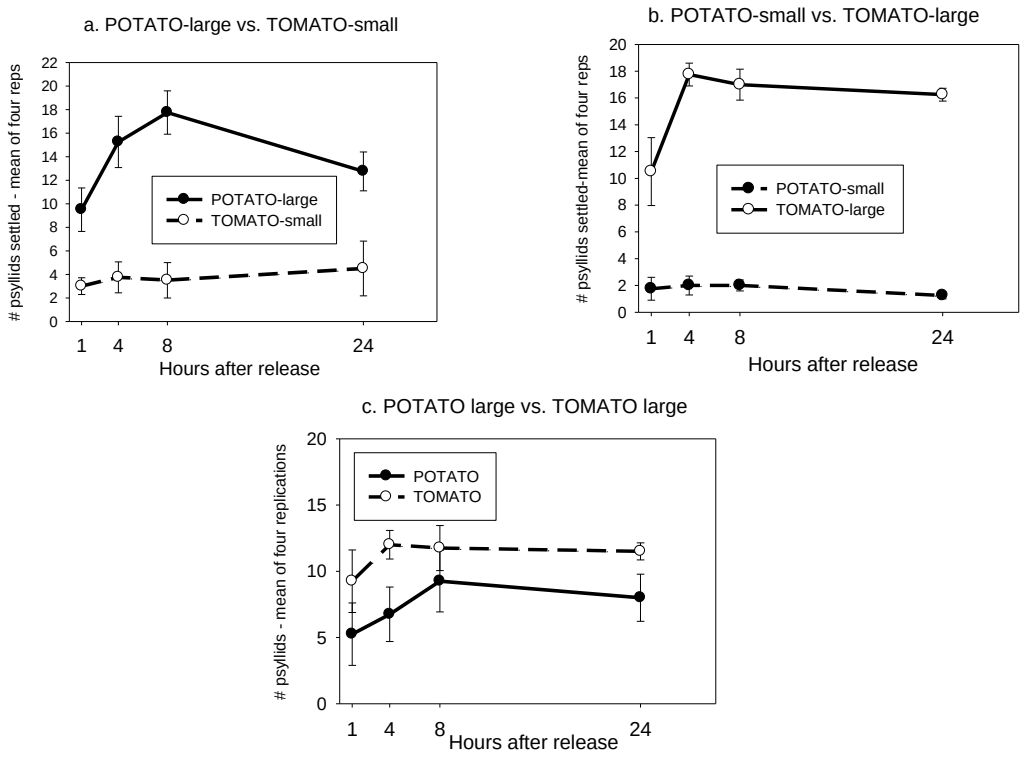
Figure 8 Plant size comparison–potato vs. tomato (large vs. small)



Graphs illustrate adult psyllid settling on the individual hosts in each pair over 24h period. Observations were recorded at 1, 4, 8 and 24h after initial psyllid release

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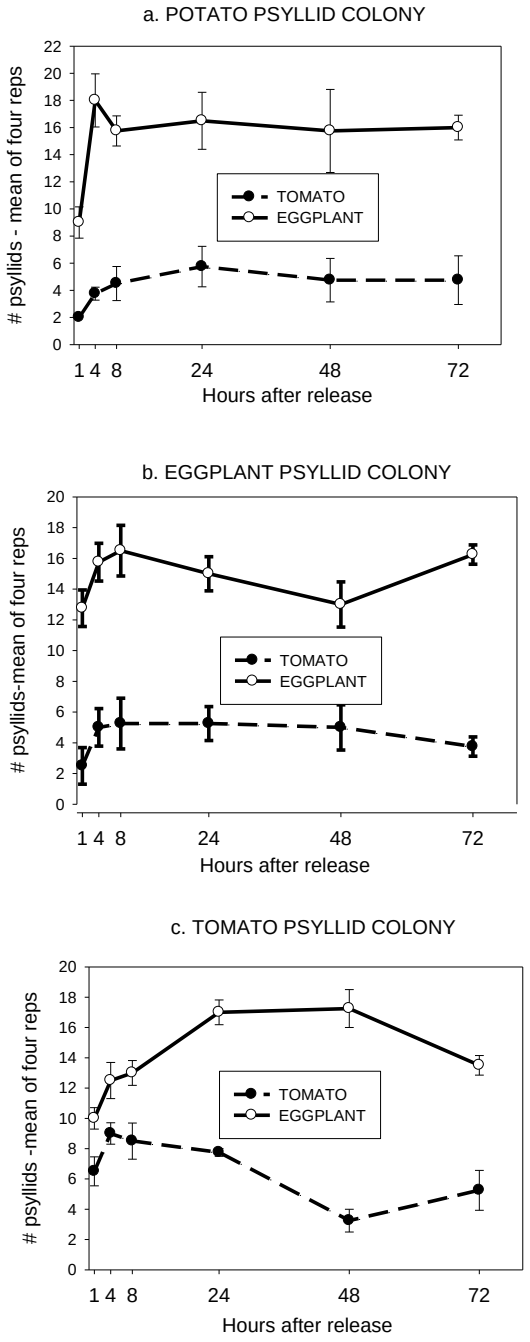
Figure 9 Host pre-adaptation – potato, eggplant and tomato colony psyllids for preference on tomato and eggplant



Graphs illustrate adult psyllid settling on the individual hosts in each pair over a 72h period. Observations were recorded at 1, 4, 8, 24, 48 and 72h after initial psyllid release

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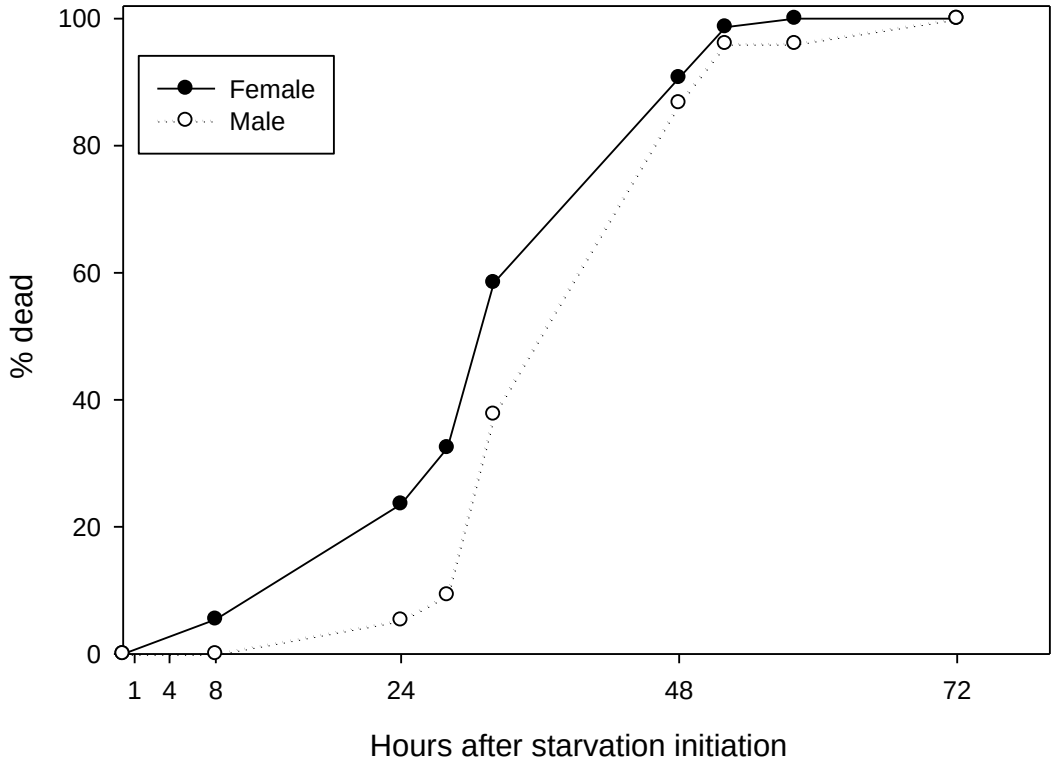
Figure 10 Starvation test – Percent mortality of male and female adults



Graphs illustrate mortality of adult females and males over a period of 72h from the time of starvation initiation.

Figure 11 Survival of Lso-infected and Lso-free *B. cockerelli* immatures on potato and SLN

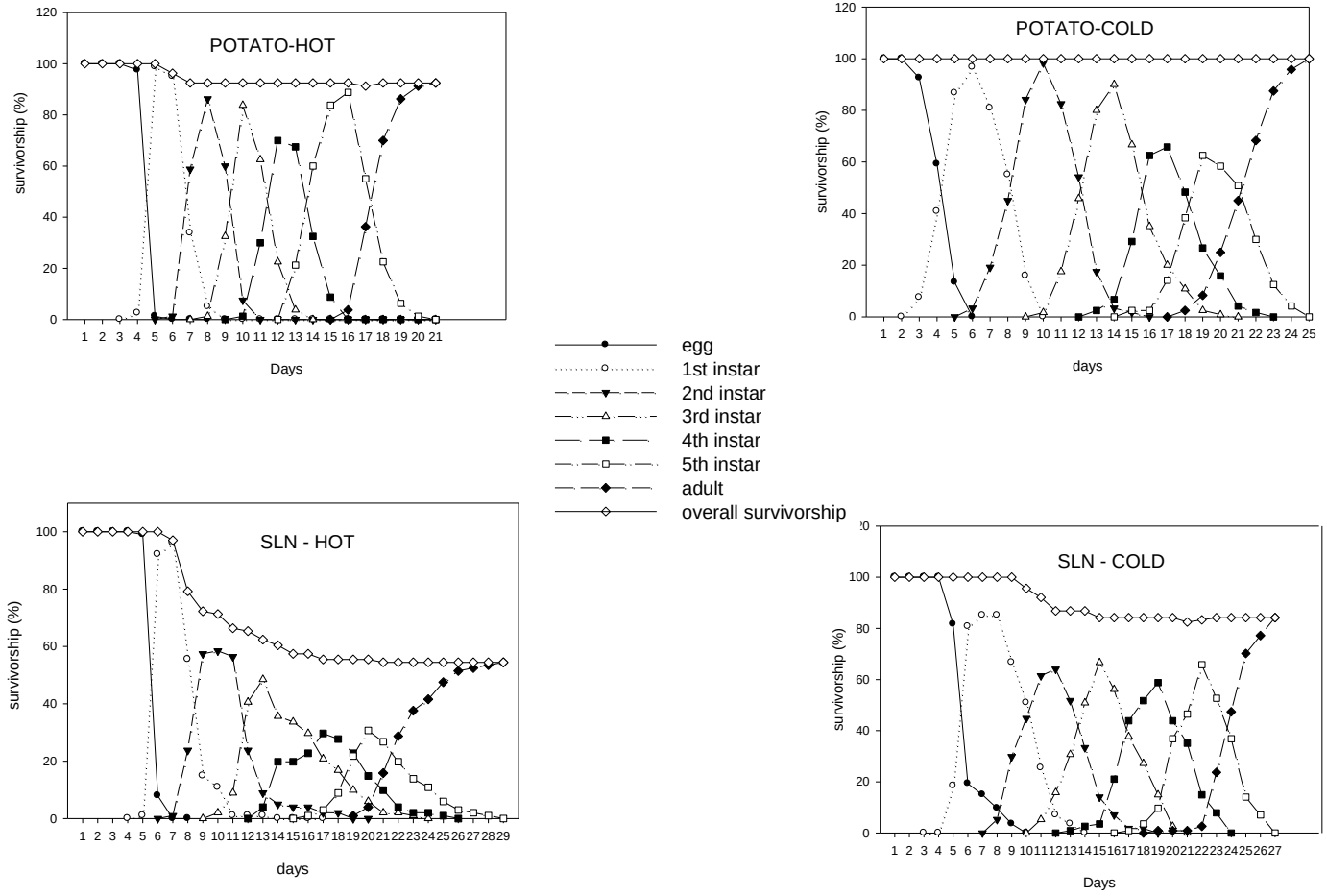


Figure 12 Age-specific survivorship (l_x), age-specific fecundity (m_x), and age-specific maternity ($l_x\ m_x$) of Lso-infective and non- infective *B. cockerelli* adults on potato and SLN

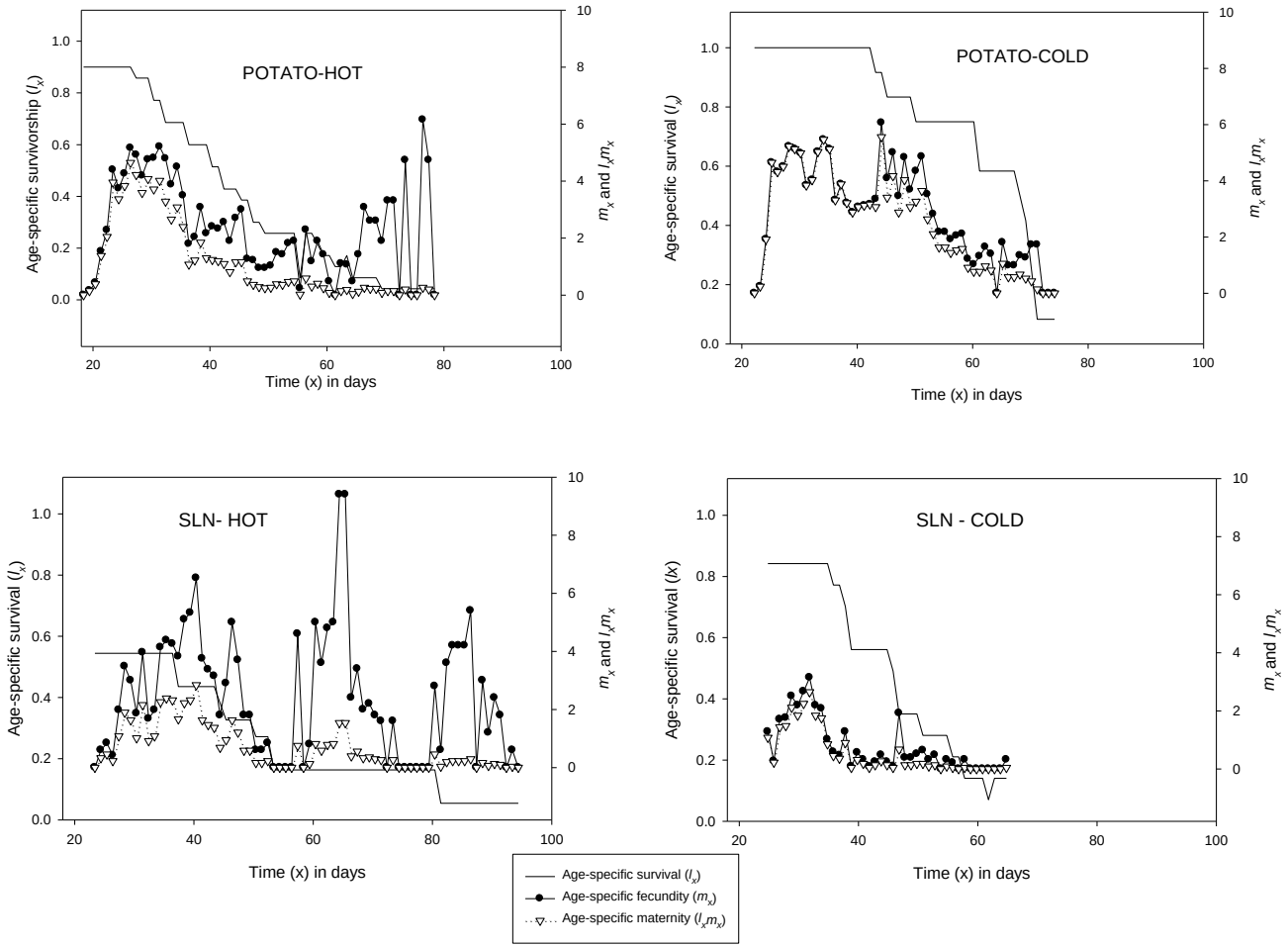


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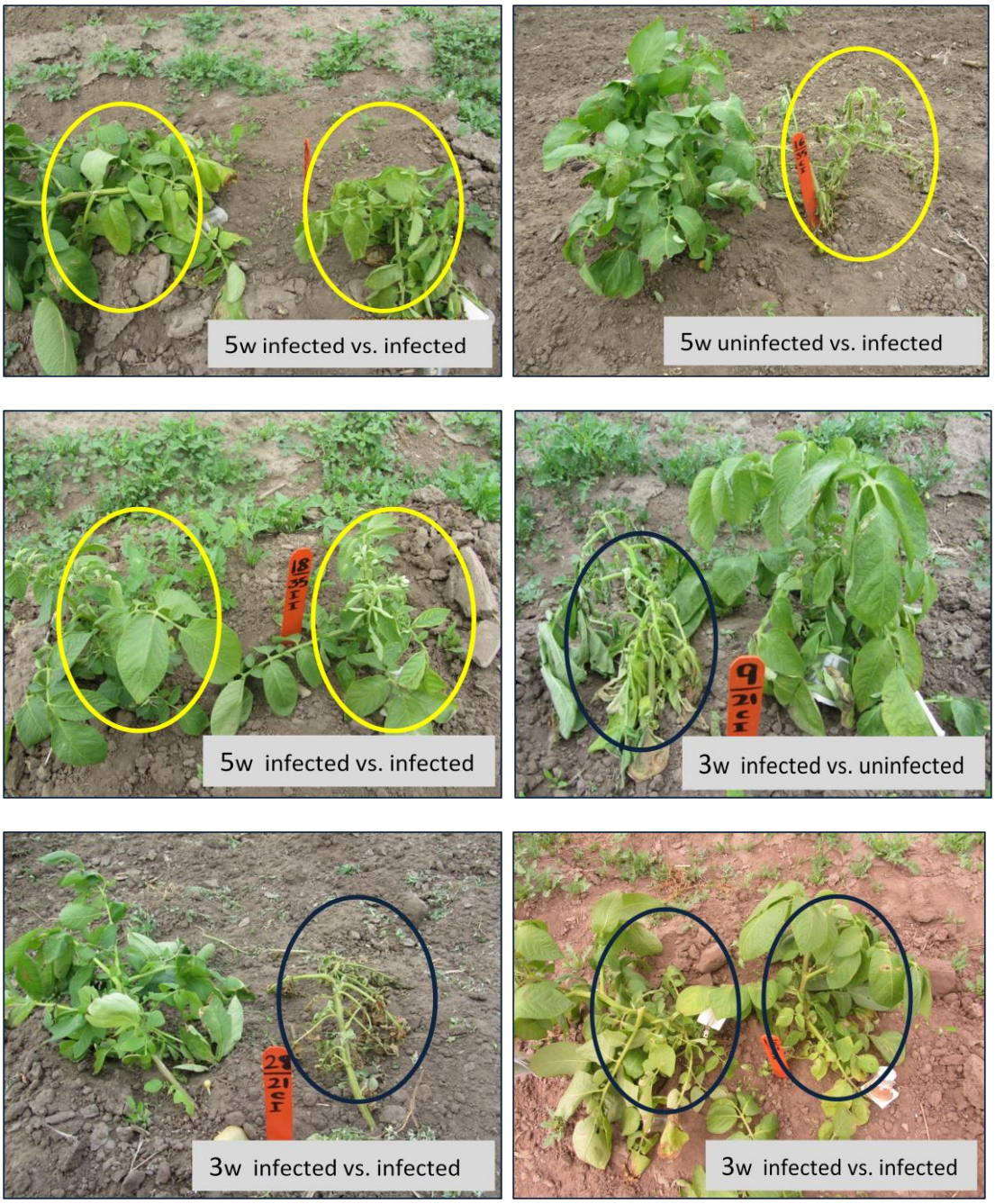
Figure 13 Variation in Lso symptom expression by Lso-infected potato plants in the field



Variation in symptom expression by individual plants could have contributed to differences in psyllid counts on infected and uninfected plants,

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Figure 14 Potato three to four weeks after Lso-infection

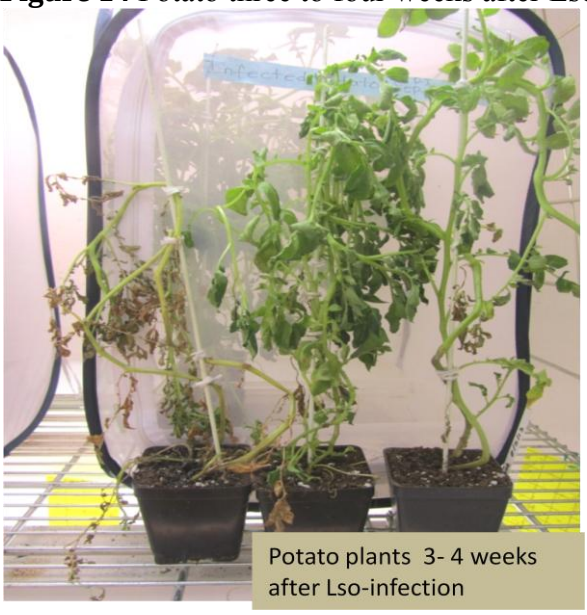

Figure 15 Tomato three, six and nine weeks after Lso-infection

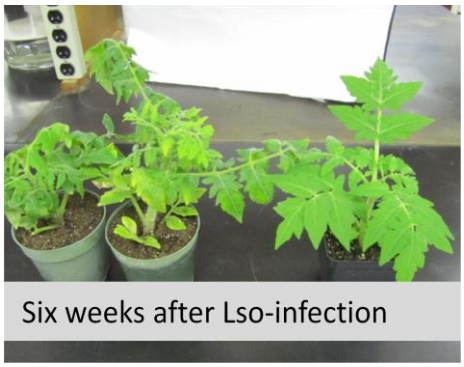

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Figure 16 Pepper six and nine weeks after Lso-infection

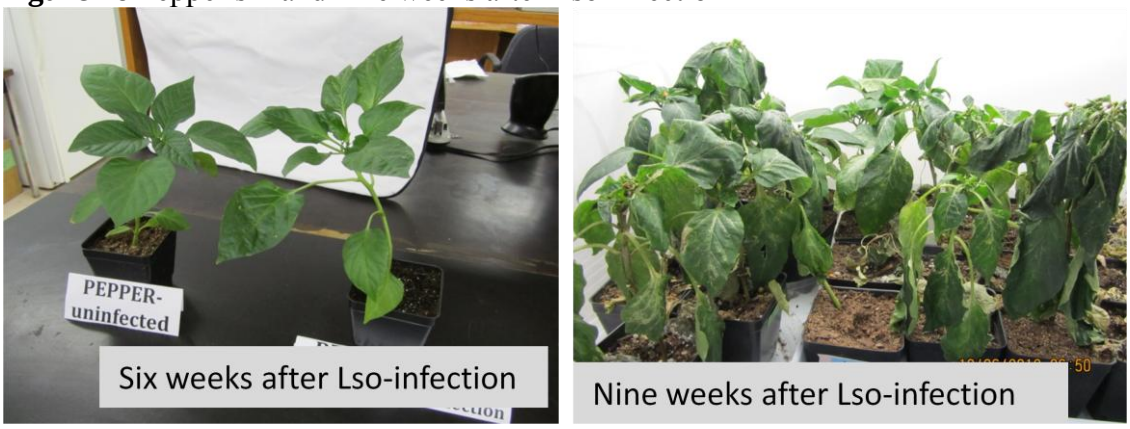


Figure 17 Eggplant three weeks after Lso-infection

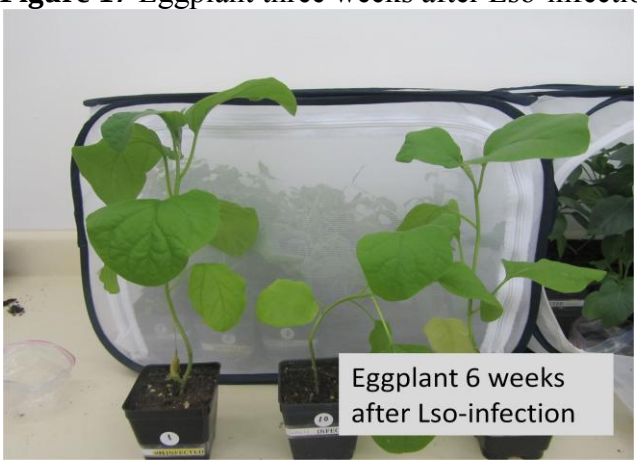


Figure 18 SLN four weeks after Lso-infection

Figure 19 Preference of resident *B. cockerelli* adults on Lso-infected and Lso-free potato 3 and 5 weeks-old when infected

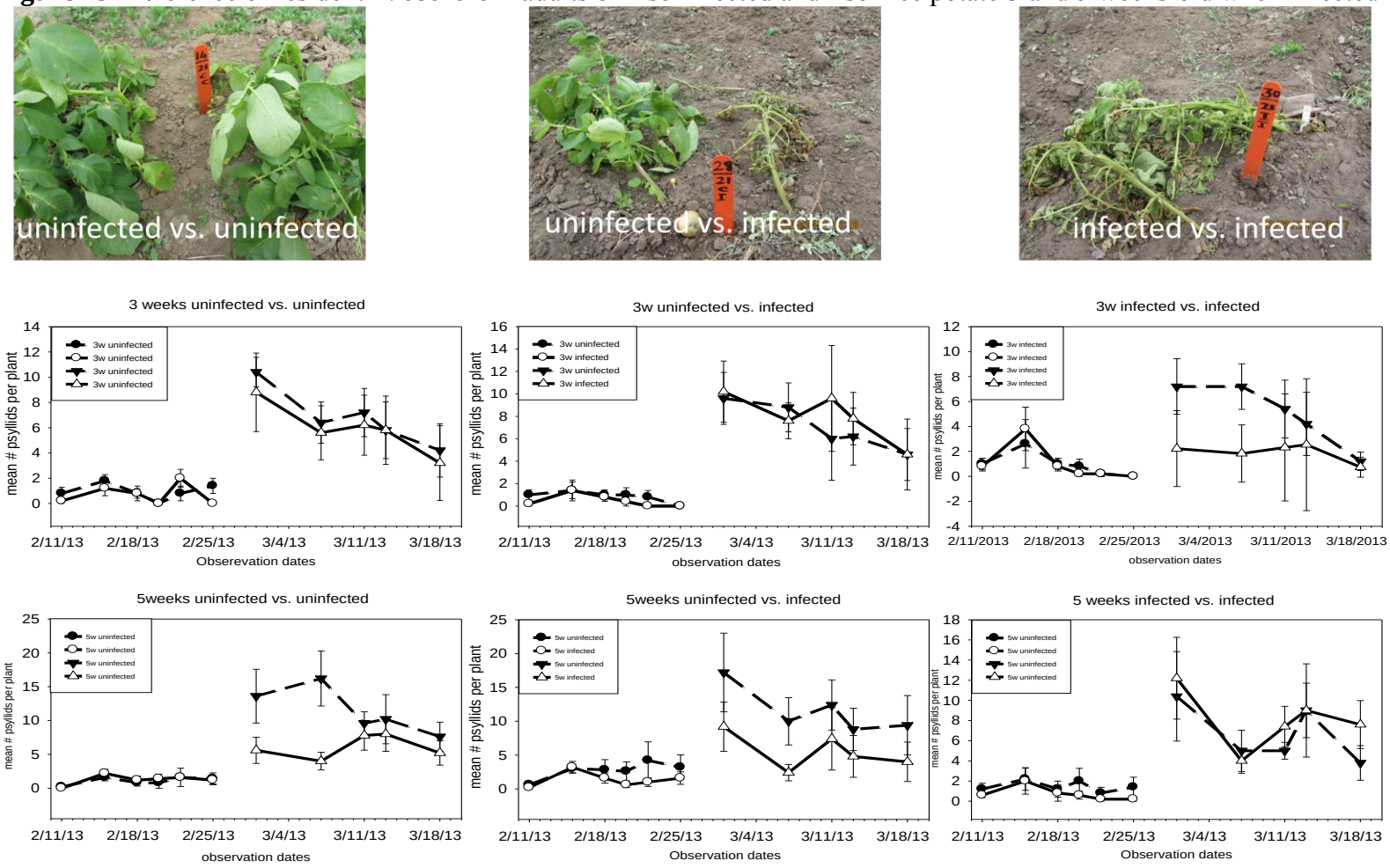
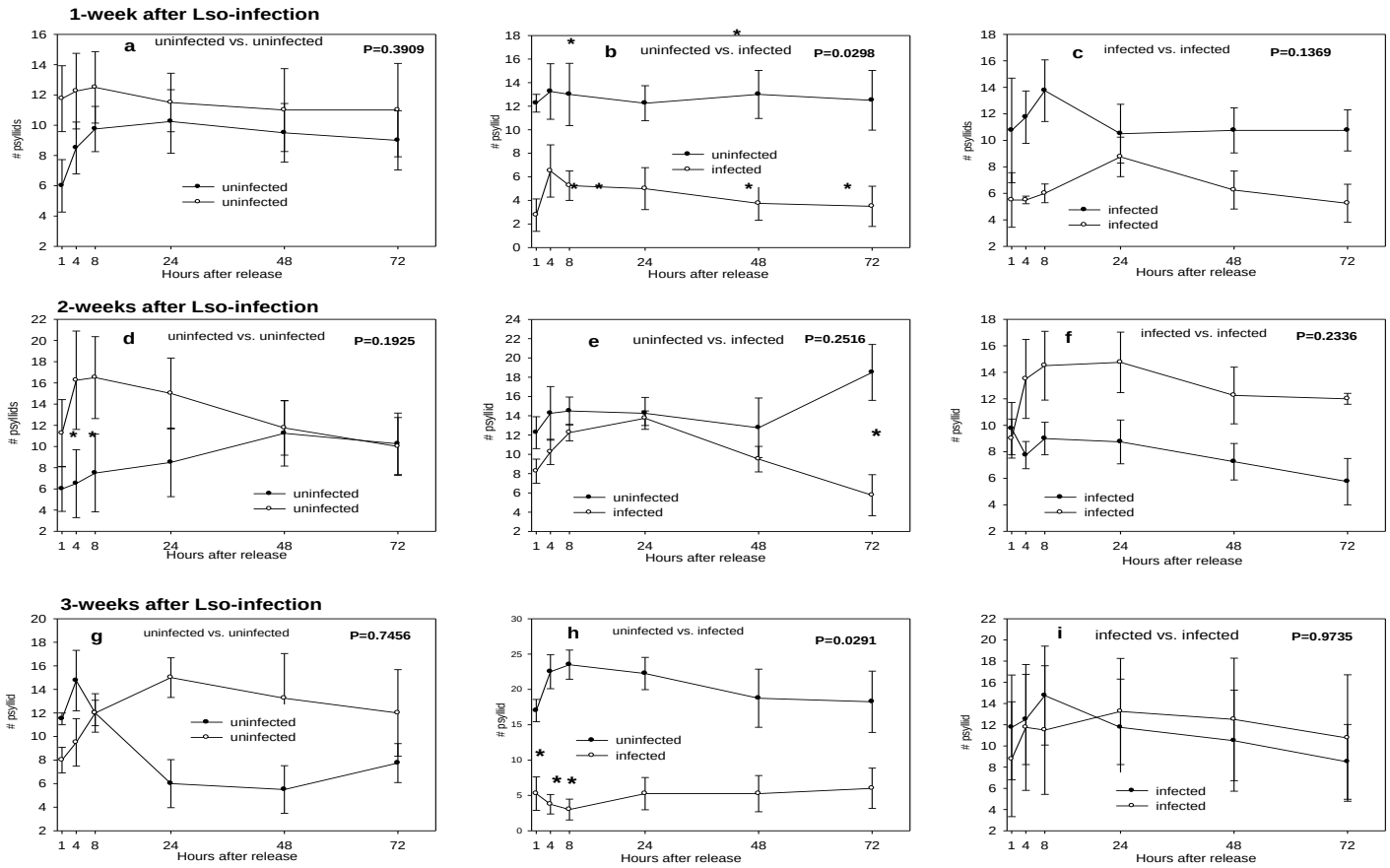
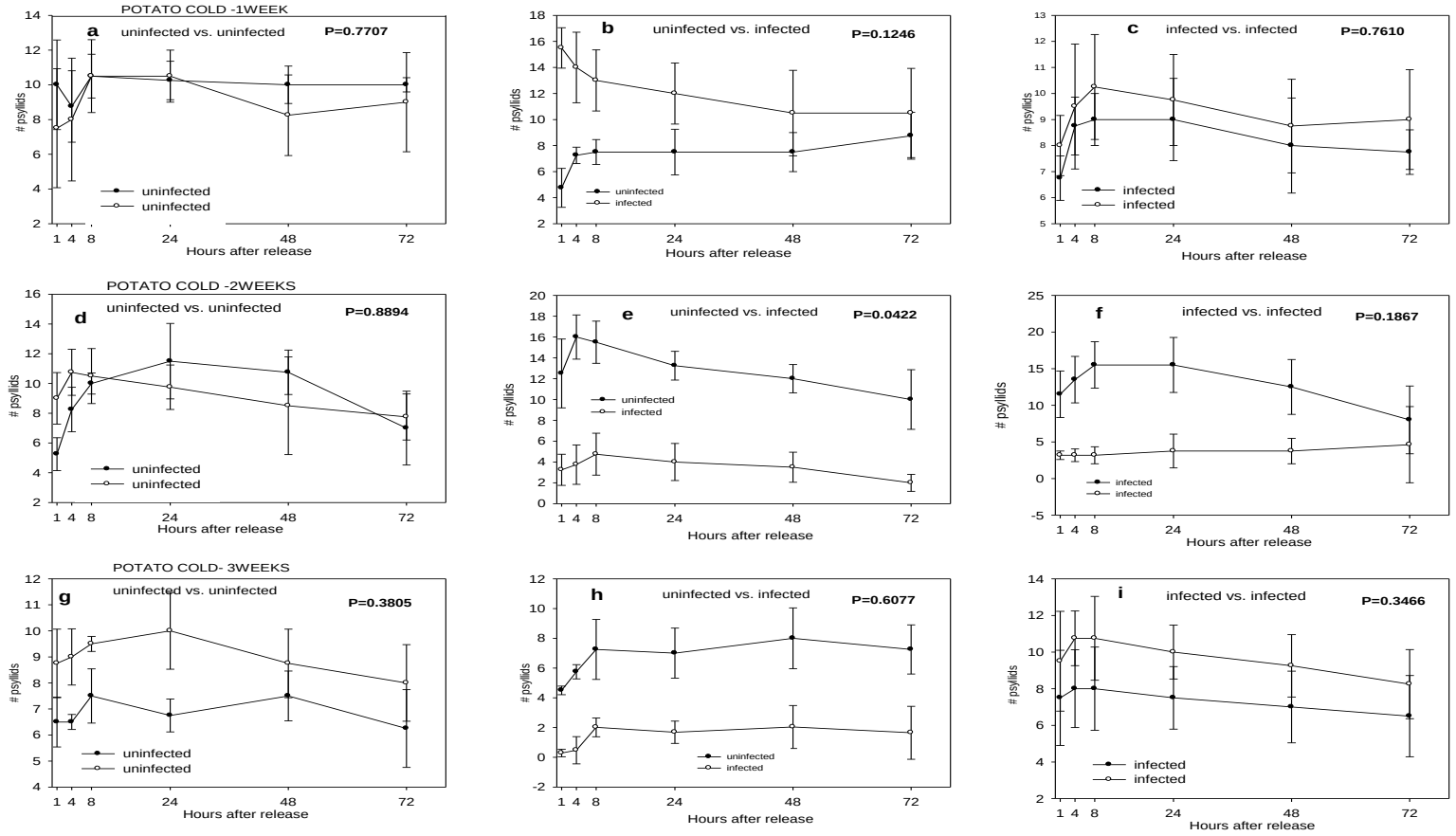


Figure 20 Settling behavior of Lso-infected *B. cockerelli* adults on potato one, two, and three weeks after Lso-infection



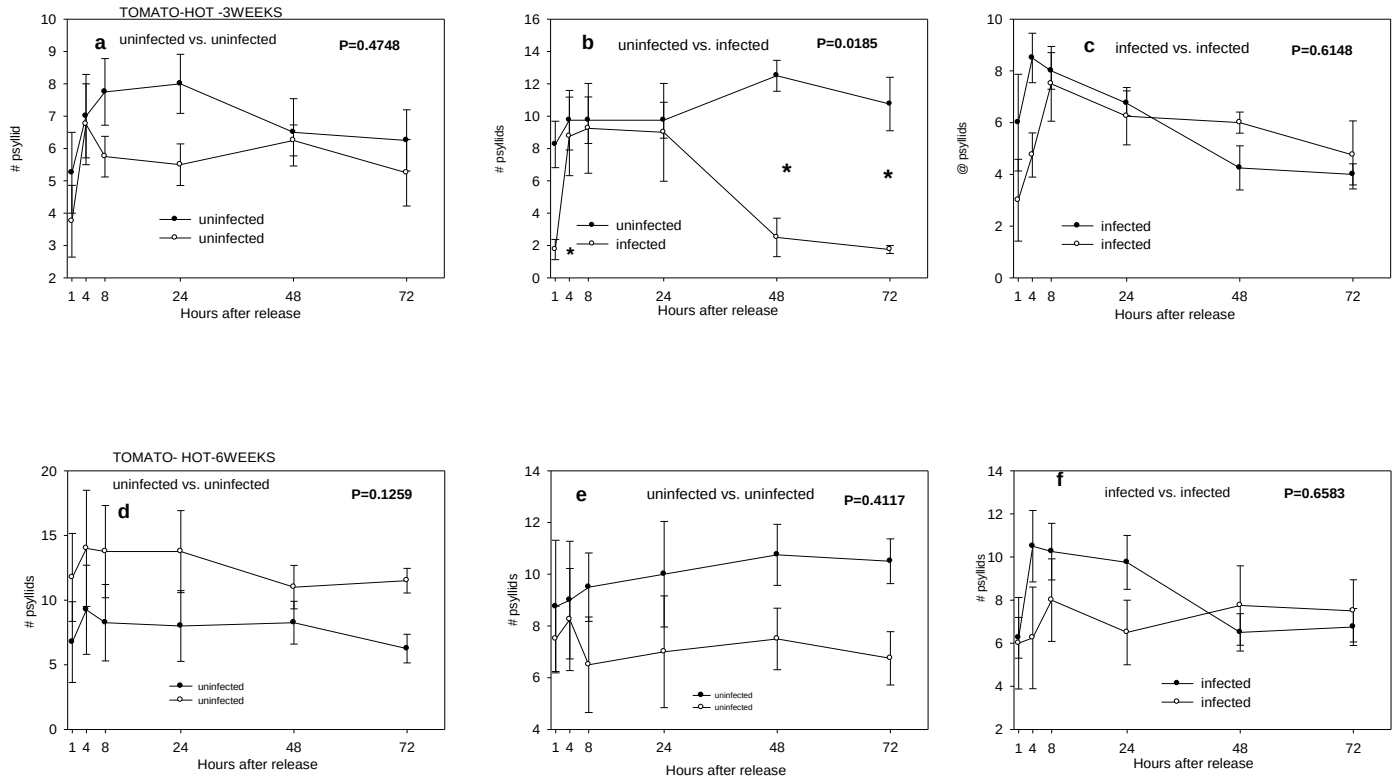
The error bars in the graphs are based on individual treatment means and standard errors. However, significant differences based on repeated measures MIXED model is represented by an asterisk.

Figure 21 Settling behavior of Lso-free *B. cockerelli* adults on potato one, two, and three weeks after Lso-infection



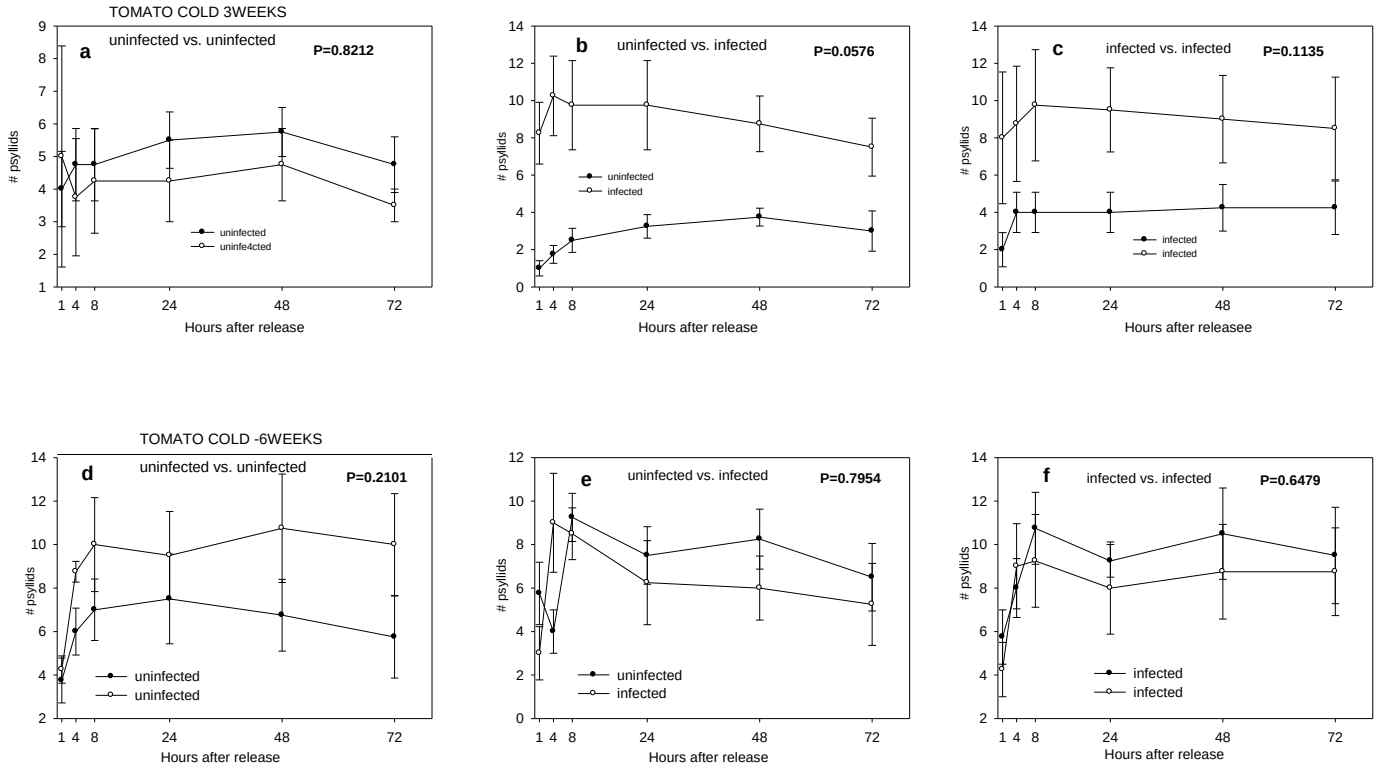
The error bars in the graphs are based on individual treatment means and standard errors. However, significant differences based on repeated measures MIXED model is represented by an asterisk.

Figure 22 Settling behavior of Lso-infected *B. cockerelli* adults on tomato, three and six weeks after Lso-infection



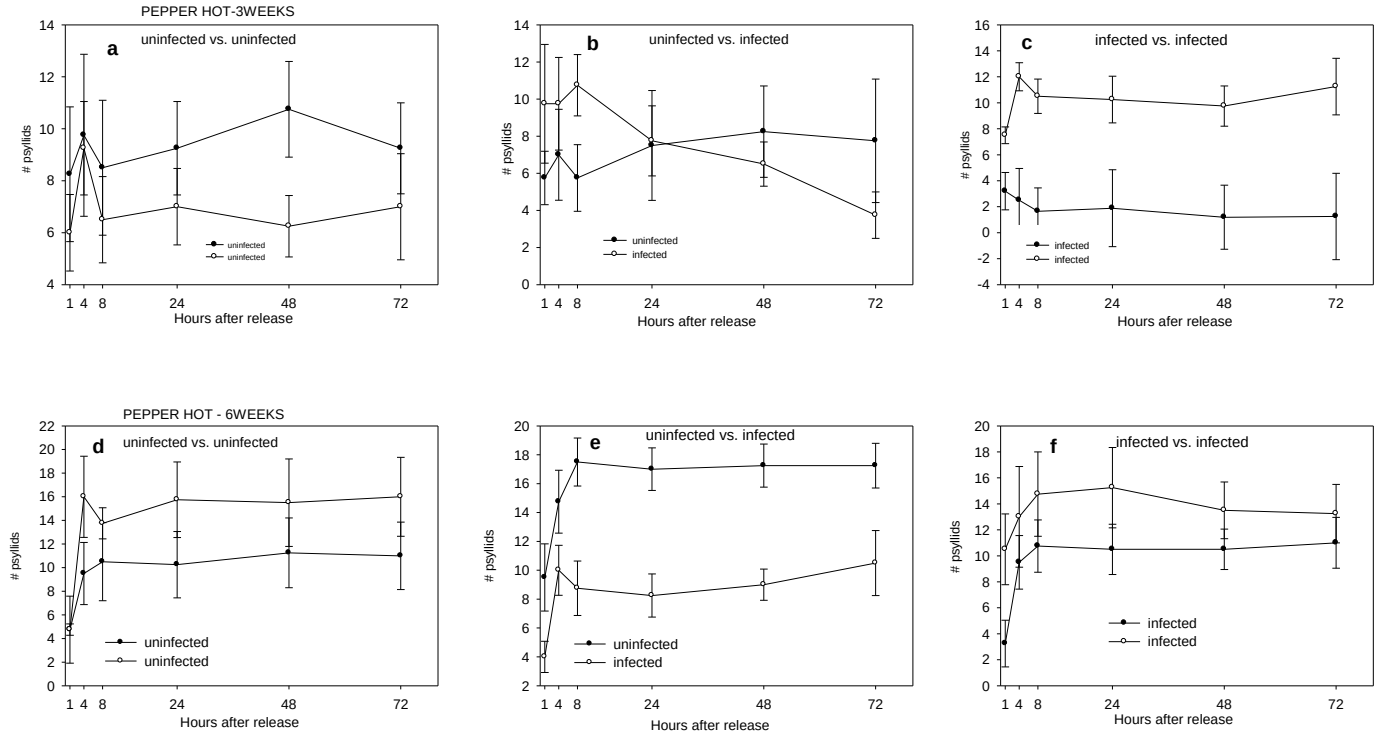
The error bars in the graphs are based on individual treatment means and standard errors. However, significant differences based on repeated measures MIXED model is represented by an asterisk.

Figure 23 Settling behavior of Lso-free *B. cockerelli* adults on tomato, three and six weeks after Lso-infection



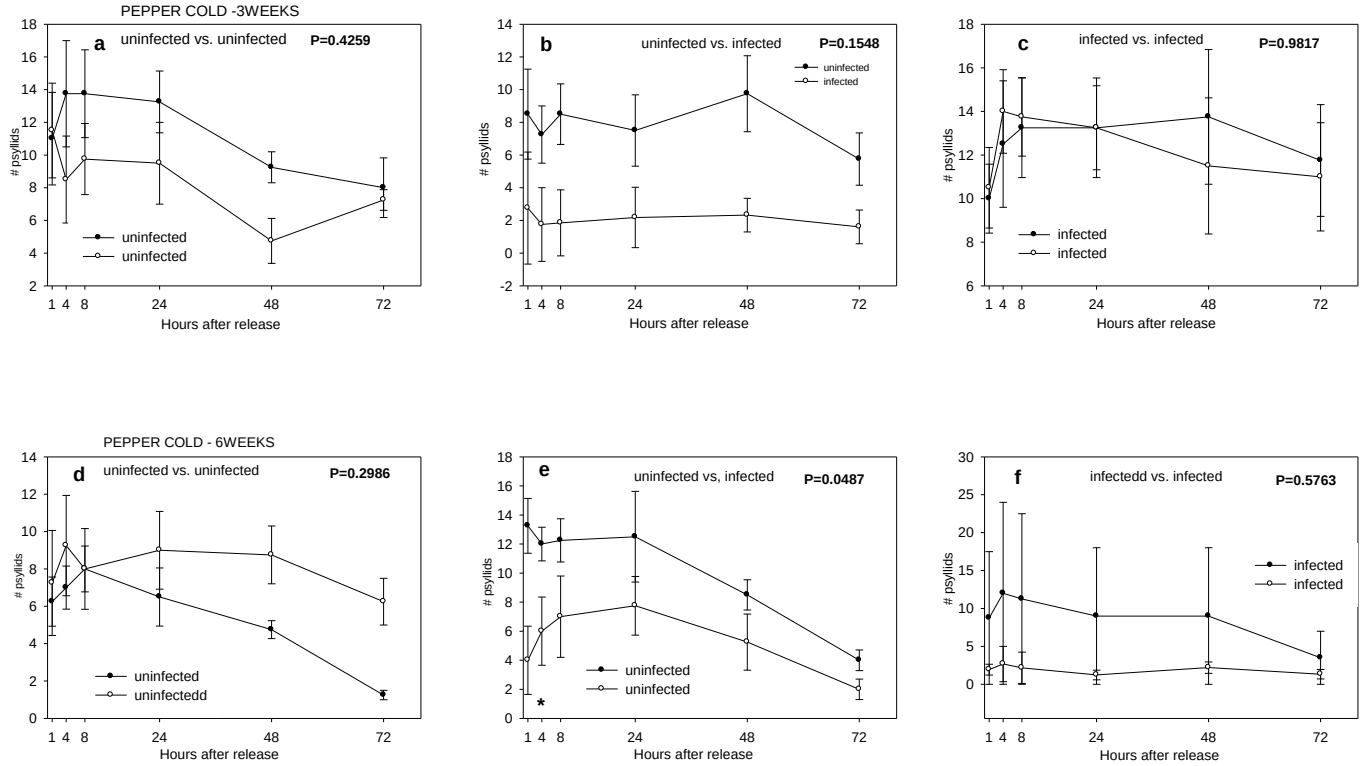
The error bars in the graphs are based on individual treatment means and standard errors. However, significant differences based on repeated measures MIXED model is represented by an asterisk.

Figure 24 Settling behavior of Lso-infected *B. cockerelli* adults on pepper, three and six weeks after Lso-infection



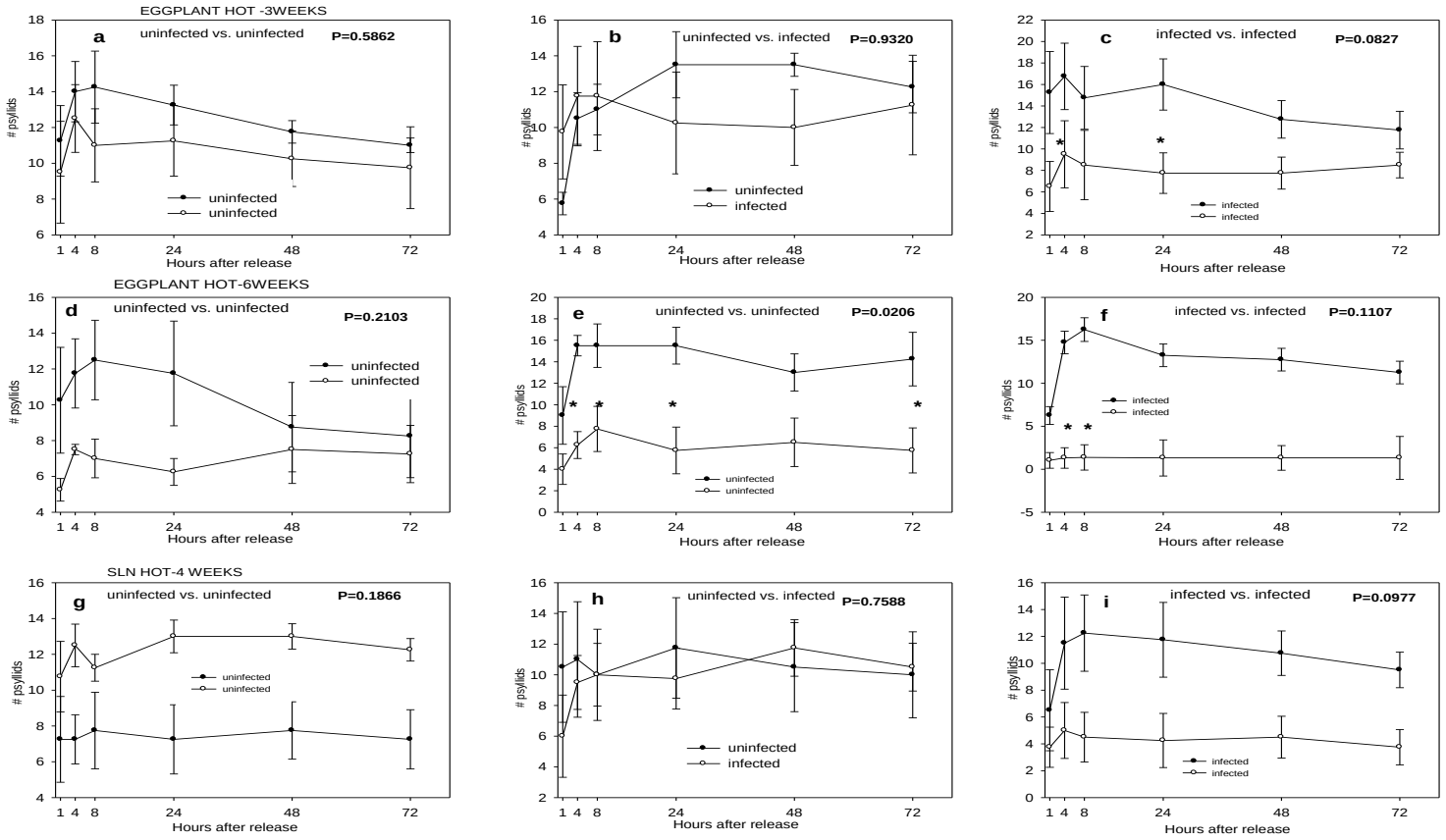
The error bars in the graphs are based on individual treatment means and standard errors. However, significant differences based on repeated measures MIXED model is represented by an asterisk.

Figure 25 Settling behavior of Lso-free *B. cockerelli* adults on pepper, three and six weeks after Lso-infection



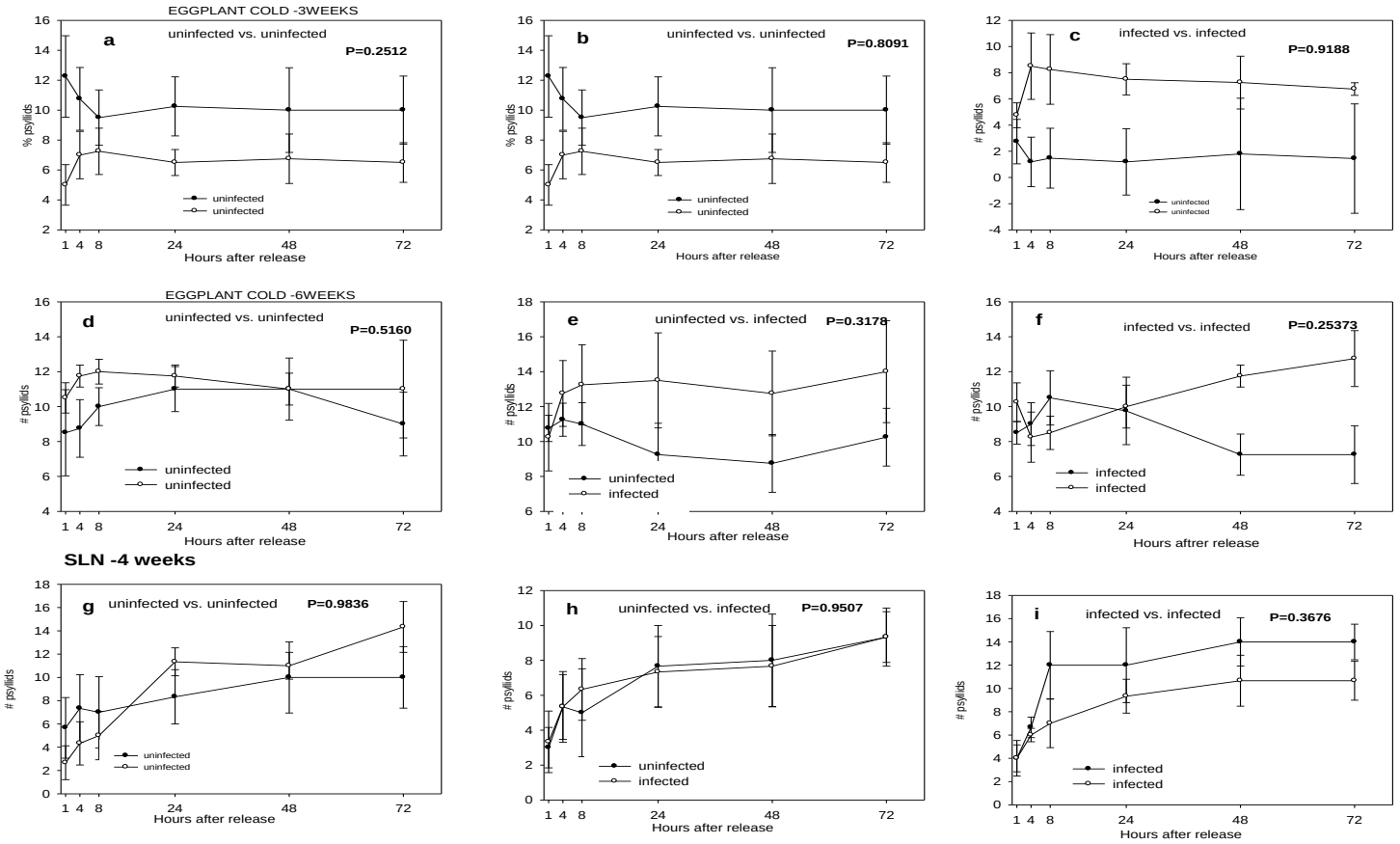
The error bars in the graphs are based on individual treatment means and standard errors. However, significant differences based on repeated measures MIXED model is represented by an asterisk.

Figure 26 Settling behavior of Lso-Infected *B. cockerelli* adults on eggplant (three and six weeks) and SLN-four weeks after Lso-infection



The error bars in the graphs are based on individual treatment means and standard errors. However, significant differences based on repeated measures MIXED model is represented by an asterisk.

Figure 27 Settling behavior of Lso-free *B. cockerelli* adults on eggplant (three and six weeks) and SLN-four weeks after Lso-infection



The error bars in the graphs are based on individual treatment means and standard errors. However, significant differences based on repeated measures MIXED model is represented by an asterisk

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Table 1 Field settling of *B. cockerelli* adults (pair-wise comparison)

Pair #	Host Pair	Trial 1 # adults	Trial 1 Settling P > |t|	Trial 2 # adults	Trial 2 Settling P > |t|
1	Potato	4.0938	0.5833	3.6923	0.3889
	Potato	3.6875		4.5000	
2	Tomato	2.2188	0.8327	2.5000	0.8529
	Tomato	2.0625		2.6731	
3	Pepper	0.3125	0.5000	2.3269	0.7110
	Pepper	0.8125		2.6731	
4	Eggplant	0.0938	0.7999	1.0769	0.5240
	Eggplant	0.2813		1.6731	
5	SLN	0.2813	0.9663	0.5000	0.7418
	SLN	0.3125		0.1923	
6	Potato	4.2188	**0.0083**	4.3846	0.2871
	Tomato	2.1875		3.3846	
7	Tomato	3.0625	**0.0018**	5.3077	**0.0004**
	Pepper	0.6250		1.7308	
8	Pepper	1.0000	0.2937	1.9038	0.5648
	Eggplant	0.2188		1.3654	
9	Eggplant	0.2188	0.7354	0.9808	0.6508
	SLN	0.4688		0.5577	
10	Potato	6.0938	**<.0001**	5.0385	**0.0003**
	Pepper	0.2813		1.4423	
11	Tomato	1.5625	0.1133	2.8269	**0.0318**
	Eggplant	0.3750		0.7692	
12	Pepper	0.4688	0.8658	2.0385	0.0528
	SLN	0.3438		0.1923	
13	Potato	3.5625	**<.0001**	6.2500	**0.0001**
	Eggplant	0.2813		2.3077	
14	Tomato	2.7813	**0.0014**	4.2115	**0.0001**
	SLN	0.2813		0.3269	
15	Potato	3.9063	**0.0005**	5.3269	**<0.0001**
	SLN	0.1563		0.4615	

The P values were obtained from SAS mixed model using repeated measures pooled across eight observation dates in Trial 1 and 13 observation dates in Trial 2. The '# adults' refers to mean number of adults from four replications and across all observation dates that settled on each plant in the host pair.

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Table 2 Abundance of *B. cockerelli* adults on plants relative to the time of day and plot density. Psyllid abundance in plots is also expressed on a per plant basis

A. Time of day

Time of day		Difference	Tukey Adj P
8am	12pm	-1.735	<.0001
8am	4pm	-0.800	0.2484
12pm	4pm	0.935	0.0057

B. Host plant plot density experiment

Plant density	Difference	Tukey Adj P
1 vs 4	-5.3036	0.1798
1 vs 16	-24.1429	<.0001
4 vs 16	-18.8393	<.0001

C. Patch density experiment- per plant basis

Plant density	Difference	Tukey Adj P
1 vs 4	5.3304	<.0001
1 vs 16	6.8114	<.0001
4 vs 16	1.4810	0.1111

The P values were obtained from SAS Mixed model using repeated measures across the seven time points. The 'difference column' shows the difference in the mean number of adults that settled on both plant densities. A positive value for the difference denotes a greater number of psyllids was observed for the first condition listed in the pairing (e.g. time point or plot density); a negative number indicates psyllids were more abundant at the second time point or density condition.

Table 3 Percentage settling of *B. cockerelli* adults on 15 host pair comparisons over a 72h period

HOST PAIR	HOST	1 HOUR	4 HOUR	8 HOUR	24 HOUR	48 HOUR	72 HOUR	POOLED ESTIMATE	SETTLING
		MEAN ± SE	MEAN ± SE	MEAN ± SE	MEAN ± SE	MEAN ± SE	MEAN ± SE		P > |t|
1	P-P	13.87 ± 4.50	23.73 ± 3.04	39.73 ± 6.10	36.80 ± 6.27	31.6 ± 8.47	32.67 ± 6.78	-0.3333	0.9161
		21.33 ± 6.96	26.59 ± 5.64	34.40 ± 6.22	38.13 ± 10.42	29.33 ± 7.85	31.87 ± 5.99		
2	T-T	11.33 ± 4.67	26.80 ± 6.96	30.13 ± 5.44	31.07 ± 4.52	33.33 ± 4.22	30.80 ± 5.16	1.6667	0.5989
		8.00 ± 4.29	20.53 ± 3.90	26.00 ± 3.23	27.87 ± 4.86	21.33 ± 1.70	25.33 ± 3.09		
3	C-C	9.6 ± 4.89	28.13 ± 5.43	29.87 ± 5.93	35.33 ± 7.20	27.60 ± 7.41	27.07 ± 4.44	-2.3333	0.4620
		25.07 ± 7.75	37.47 ± 4.66	39.33 ± 5.42	37.33 ± 5.10	34.80 ± 3.49	27.60 ± 4.99		
4	E-E	32.53 ± 12.75	47.47 ± 9.07	44.93 ± 5.82	43.20 ± 1.95	40.13 ± 3.02	34.56 ± 4.77	1.6667	0.5989
		29.47 ± 5.60	39.60 ± 7.51	37.07 ± 4.66	36.93 ± 3.98	34.40 ± 4.81	34.80 ± 3.49		
5	S-S	22.93 ± 4.29	31.87 ± 6.63	36.67 ± 9.60	36.53 ± 13.26	25.87 ± 9.43	24.53 ± 10.75	1.5333	0.6284
		16.27 ± 3.99	28.80 ± 4.53	31047 ± 4.87	24.00 ± 5.10	23.87 ± 6.47	27.20 ± 7.81		
6	P-T	23.47 ± 4.60	42.53 ± 4.05	47.20 ± 4.60	41.20 ± 4.58	34.80 ± 7.67	34.13 ± 8.22	4.0000	0.2092
		20.67 ± 4.64	21.07 ± 3.20	22.53 ± 4.42	27.20 ± 2.32	25.87 ± 3.46	26.53 ± 3.56		
7	C-T	18.67 ± 2.26	44.67 ± 6.02	53.33 ± 8.37	51.47 ± 9.52	49.73 ± 11.05	44.67 ± 10.14	6.7667 *	**0.0357**
		8.67 ± 4.78	20.00 ± 9.37	20.27 ± 7.71	23.47 ± 8.68	22.80 ± 7.55	18.80 ± 6.47		
8	C-E	20.80 ± 4.42	33.73 ± 7.24	38.53 ± 8.08	33.73 ± 6.92	35.73 ± 7.56	31.07 ± 11.33	-1.6333	0.6062
		30.13 ± 8.78	35.73 ± 8.25	38.13 ± 5.94	35.67 ± 6.15	38.93 ± 6.60	39.87 ± 6.87		
9	E-S	31.47 ± 4.23	56.67 ± 11.93	55.33 ± 10.93	53.33 ± 10.33	48.40 ± 10.51	45.47 ± 8.85	9.200 *	**0.0049**
		15.60 ± 4.90	14.93 ± 5.13	20.00 ± 4.22	18.00 ± 4.67	16.13 ± 4.58	18.27 ± 5.13		
10	P-C	29.47 ± 11.06	39.73 ± 13.14	47.07 ± 7.05	45.87 ± 6.50	45.33 ± 5.44	48.67 ± 3.89	4.1667	0.1911
		20.67 ± 7.70	36.80 ± 14.12	34.80 ±11.60	32.67 ± 9.21	25.87 ± 7.80	22.27 ± 9.29		
11	T-E	9.33 ± 5.10	18.00 ± 5.01	12.40 ± 4.27	13.07 ± 4.09	13.73 ± 4.02	11.33 ± 3.27	-11.5000	**0.0005**
		38.13 ± 9.06	54.67 ± 7.72	58.27 ± 6.17	58.40 ± 4.38	53.73 ± 6.99	51.60 ± 3.96		
12	S-C	27.20 ± 9.78	36.00 ± 9.97	38.27 ± 9.27	30.13 ± 6.72	21.07 ± 4.43	28.67 ± 5.73	-2.6667	0.4008
		25.87 ± 8.27	44.53 ± 11.34	45.60 ± 13.05	46.27 ± 11.05	43.07 ± 8.81	36.93 ± 9.62		
13	P-E	24.53 ± 5.97	28.67 ± 9.58	34.53 ± 8.93	37.87 ± 9.54	29.20 ± 10.60	27.60 ± 9.51	-1.7667	0.5772
		31.73 ± 8.16	42.13 ± 9.04	40.80 ± 5.12	35.07 ± 7.59	34.93 ± 8.01	34.93 ± 8.01		

Table 3 continued. Percentage settling of *B. cockerelli* adults on 15 host pair comparisons over a 72h period

HOST PAIR	HOST	1 HOUR	4 HOUR	8 HOUR	24 HOUR	48 HOUR	72 HOUR	POOLED ESTIMATE	SETTLING
		MEAN ± SE	MEAN ± SE	MEAN ± SE	MEAN ± SE	MEAN ± SE	MEAN ± SE		P > |t|
14	T-S	15.47 ± 4.70	25.47 ± 9.62	33.20 ± 10.06	27.87 ± 7.40	24.93 ± 8.06	22.40 ± 6.20	-3.1000	0.3292
		19.73 ± 6.66	32.93 ± 7.58	43.73 ± 5.45	41.60 ± 6.66	39.73 ± 5.76	37.07 ± 7.74		
15	S-P	17.60 ± 6.72	19.60 ± 7.70	20.40 ± 9.52	25.60 ± 11.80	26.27 ± 11.74	21.07 ± 9.36	-6.3667	**0.0477**
		33.20 ± 8.62	44.40 ± 9.07	47.47 ± 7.47	46.13 ± 9.02	38.53 ± 9.41	48.00 ± 10.25		

The numbers indicate percentage of adult *B. cockerelli* settling on the individual host plants ± SE. The P values were obtained from a SAS mixed model using repeated measures ANOVA across the six time points. The presented P value was pooled across all time points and mean of five replications. The estimate shows the differences in the mean number of adults that settled on both plants in the host pair. A positive value of the estimate denotes the first host had a greater mean number of psyllids and a negative value denotes the second host had a greater mean number of psyllids. The 'same host' pairs are abbreviated as follows: P-P for potato-potato , T-T for tomato-tomato, C-C for pepper-pepper, E-E for eggplant-eggplant and S-S for SLN-SLN. All possible combinations of 'mixed host' pairs were included.

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Table 4 Ovipositional preference of *B. cockerelli* on 15 host pairs in a paired choice test

Host pair	Host	No. of eggs laid	Oviposition P > |t|
1	P-P	P1 52.20 ± 25.35 P2 85.00 ± 35.45	0.4350
2	T-T	T1 47.83 ± 21.73 T2 19.75 ± 8.54	0.5609
3	C-C	C1 91.80 ± 26.22 C2 79.20 ± 20.92	0.7637
4	E-E	E1 125.6 ± 21.90 E2 99.80 ± 47.04	0.5387
5	S-S	S1 78.20 ± 54.91 S2 55.20 ± 15.82	0.5836
6	P-T	P1 58.80 ± 26.93 T2 65.40 ± 19.43	0.8749
7	C-T	C1 149.2 ± 57.66 T1 23.80 ± 6.52	**0.0039**
8	C-E	C1 110.20 ± 22.13 E2 93.60 ± 36.97	0.6922
9	E-S	E1187.6 ± 28.13 S2 86.20 ± 24.35	**0.0182**
10	P-C	P1 83.00 ± 28.18 C2 59.40 ± 13.87	0.5738
11	T-E	T1 38.60 ± 20.89 E2 142.20 ± 55.96	**0.0159**
12	S-C	S1 28.20 ± 13.15 C2 81.80 ± 21.36	0.2040
13	P-E	P1 37.40 ± 7.39 E2 91.40 ± 35.68	0.2007
14	T-S	T1 77.80 ± 43.85 S2 108.2 ± 32.86	0.4691
15	S-P	S1 82.00 ± 38.03 P2 84.20 ± 16.16	0.9581

The P values were obtained from SAS mixed model and mean of eggs ± SE on both plants in the host pair for a mean of five replications. The 'same host' pairs are abbreviated as follows: P-P for potato-potato, T-T for tomato-tomato, C-C for pepper-pepper, E-E for eggplant-eggplant and S-S for SLN-SLN. All possible combinations of 'mixed host' pairs were included.

Table 5 Life history of Lso-infected and Lso-free *B. cockerelli* on potato and SLN

Parameters	POTATO-HOT			SLN-HOT			DF	t-value	Tukey adj
	mean ± SE	min	max	mean ± SE	min	max			Pr> |t|
egg period	4.00 ± 0.02	3.00	5.00	5.05 ± 0.04	4.00	6.00	43.40	-3.62	0.0043
1st instar	2.41 ± 0.07	1.00	4.00	2.82 ± 0.12	2.00	5.00	46.10	-1.340	0.5425
2nd instar	2.31 ± 0.06	1.00	3.00	3.64 ± 0.21	2.00	10.00	42.80	-4.660	0.0002
3rd instar	2.23 ± 0.05	2.00	3.00	4.35 ± 0.28	2.00	9.00	41.30	-6.540	<0.0001
4th instar	2.27 ± 0.05	2.00	3.00	3.29 ± 0.18	1.00	6.00	41.30	-4.790	0.0002
5th instar	3.68 ± 0.07	2.00	5.00	2.73 ± 0.10	2.00	4.00	49.20	5.230	<0.0001
total days	16.89 ± 0.12	15.00	20.00	21.87 ± 0.29	18.00	28.00	43.90	-11.290	<0.0001
nymph days	12.89 ± 0.11	11.00	16.00	16.82 ± 0.30	13.00	23.00	44.20	-8.140	<0.0001
Longevity (days)	28.76 ± 3.05	10.00	62.00	35.60 ± 6.49	15.00	73.00	45.40	-1.440	0.4809
Pre-oviposition	4.62 ± 0.36	1.00	7.00	4.00 ± 0.86	1.00	9.00	47.00	0.730	0.8847
Oviposition	21.29 ± 3.13	5.00	56.00	33.30 ± 6.72	11.00	68.00	51.00	-2.170	0.1445
# eggs	164.57 ± 22.42	43.00	369.00	150.10 ± 26.60	50.00	318.00	51.00	0.410	0.9769
Parameters	POTATO-COLD			SLN-COLD			DF	t-value	Tukey adj
	mean ± SE	min	max	mean ± SE	min	max			Pr> |t|
egg period	3.65 ± 0.07	2.00	5.00	5.27 ± 0.13	4.00	10.00	39.00	-6.130	<0.0001
1st instar	3.83 ± 0.09	2.00	6.00	4.20 ± 0.13	2.00	7.00	37.50	-1.360	0.5300
2nd instar	4.09 ± 0.11	2.00	7.00	3.54 ± 0.12	2.00	7.00	32.60	2.190	0.1454
3rd instar	3.73 ± 0.11	2.00	7.00	3.68 ± 0.12	2.00	7.00	32.10	0.070	0.9999
4th instar	2.63 ± 0.06	2.00	5.00	3.38 ± 0.07	2.00	5.00	30.10	-4.570	0.0003
5th instar	2.76 ± 0.07	2.00	4.00	3.27 ± 0.06	2.00	6.00	36.10	-3.440	0.0069
total days	20.69 ± 0.14	17.00	24.00	23.33 ± 0.14	18.00	26.00	35.90	-6.470	<0.0001
nymph days	17.04 ± 0.15	13.00	21.00	18.06 ± 0.16	13.00	21.00	37.00	-2.280	0.1197
Longevity (days)	41.83 ± 3.18	22.00	63.00	25.00 ± 3.00	12.00	42.00	42.00	3.100	0.0172
Pre-oviposition	3.17 ± 0.70	1.00	10.00	3.08 ± 0.43	0.00	5.00	44.50	0.080	0.9998
Oviposition	34.17 ± 3.12	20.00	60.00	18.17 ± 2.87	7.00	40.00	51.00	2.720	0.0424
# eggs	227.58 ± 34.29	81.00	470.00	41.08 ± 6.39	15.00	91.00	51.00	4.940	<0.0001

Table 5 continued. Life history of Lso-infected and Lso-free *B. cockerelli* on potato and SLN

Parameters	POTATO-HOT			POTATO-COLD			DF	t-value	Tukeyadj Pr> |t|
	mean ± SE	min	max	mean ± SE	min	max			
egg period	4.00 ± 0.02	3.00	5.00	3.65 ± 0.07	2.00	5.00	40.30	1.310	0.5634
1st instar	2.41 ± 0.07	1.00	4.00	3.83 ± 0.09	2.00	6.00	39.70	-5.110	<0.0001
2nd instar	2.31 ± 0.06	1.00	3.00	4.09 ± 0.11	2.00	7.00	35.00	-6.540	<0.0001
3rd instar	2.23 ± 0.05	2.00	3.00	3.73 ± 0.11	2.00	7.00	34.30	-4.730	0.0002
4th instar	2.27 ± 0.05	2.00	3.00	2.63 ± 0.06	2.00	5.00	32.50	-2.020	0.2004
5th instar	3.68 ± 0.07	2.00	5.00	2.76 ± 0.07	2.00	4.00	39.00	5.890	<0.0001
total days	16.89 ± 0.12	15.00	20.00	20.69 ± 0.14	17.00	24.00	38.00	-9.040	<0.0001
nymph days	12.89 ± 0.11	11.00	16.00	17.04 ± 0.15	13.00	21.00	38.90	-8.820	<0.0001
Longevity (days)	28.76 ± 3.05	10.00	62.00	41.83 ± 3.18	22.00	63.00	46.40	-2.640	0.0535
Pre-oviposition	4.62 ± 0.36	1.00	7.00	3.17 ± 0.70	1.00	10.00	47.60	1.990	0.2068
Oviposition	21.29 ± 3.13	5.00	56.00	34.17 ± 3.12	20.00	60.00	51.00	-2.470	0.0763
# eggs	164.57 ± 22.42	43.00	369.00	227.58 ± 34.29	81.00	735.00	51.00	-1.880	0.2474

Parameters	SLN-HOT			SLN-COLD			DF	t-value	Tukeyadj Pr> |t|
	mean ± SE	min	max	mean ± SE	min	max			
egg period	5.05 ± 0.04	4.00	6.00	5.27 ± 0.13	4.00	10.00	42.40	-0.730	0.8845
1st instar	2.82 ± 0.12	2.00	5.00	4.20 ± 0.13	2.00	7.00	44.40	-4.720	0.0002
2nd instar	3.64 ± 0.21	2.00	10.00	3.54 ± 0.12	2.00	7.00	40.90	0.650	0.9148
3rd instar	4.35 ± 0.28	2.00	9.00	3.68 ± 0.12	2.00	7.00	39.50	2.390	0.0978
4th instar	3.29 ± 0.18	1.00	6.00	3.38 ± 0.07	2.00	5.00	39.40	-0.920	0.7921
5th instar	2.73 ± 0.10	2.00	4.00	3.27 ± 0.06	2.00	6.00	47.00	-3.010	0.0219
total days	21.87 ± 0.29	18.00	28.00	23.33 ± 0.14	18.00	26.00	42.30	-2.830	0.0354
nymph days	16.82 ± 0.30	13.00	23.00	18.06 ± 0.16	13.00	21.00	42.70	-2.110	0.1664
Longevity (days)	35.60 ± 6.49	15.00	73.00	25.00 ± 3.00	12.00	42.00	42.80	2.000	0.2074
Pre-oviposition	4.00 ± 0.86	1.00	9.00	3.08 ± 0.43	0.00	5.00	45.50	1.110	0.6850
Oviposition	33.30 ± 6.72	11.00	68.00	18.17 ± 2.87	7.00	40.00	51.00	2.460	0.0793
# eggs	150.10 ± 26.60	50.00	318.00	41.08 ± 6.39	15.00	91.00	51.00	2.760	0.0392

Table 6 Survival of Lso-infected and Lso-free *B. cockerelli* nymphs on potato and SLN

Instars	POTATO-HOT mean ± SE	SLN-HOT mean ± SE	Tukey adj Pr> |t|
1st instar	94.00 ± 3.40	70.61 ± 4.04	<.0001
2nd instar	100.00 ± 0.00	84.27 ± 4.63	0.0007
3rd instar	100.00 ± 0.00	93.49 ± 3.48	0.0378
4th instar	100.00 ± 0.00	99.09 ± 0.91	0.4665
5th instar	100.00 ± 0.00	100 00 ± 0.00	1.0000
1st - 5th	94.00 ± 3.40	54.89 ± 4.70	<.0001
Instars	POTATO-COLD mean ± SE	SLN-COLD mean ± SE	Tukey adj Pr> |t|
1st instar	100.00 ± 0.00	91.82 ± 3.77	0.0660
2nd instar	100.00 ± 0.00	92.45 ± 3.72	0.1232
3rd instar	100.00 ± 0.00	100.00 ± 0.00	1.0000
4th instar	100.00 ± 0.00	100.00 ± 0.00	0.4464
5th instar	100.00 ± 0.00	100.00 ± 0.00	1.0000
1st - 5th	100.00 ± 0.00	84.67 ± 4.70	0.0017

Table 7 Life table parameters of Lso-infected and Lso-free *B. cockerelli* on potato and SLN

Parameter	POTATO-HOT		POTATO-COLD	
	True calculation	Jackknife estimate (95% CL)	True calculation	Jackknife estimate (95% CL)
Σm_x	142.33		149.87	
r_m	0.15183	0.15150 (0.13607 - 0.16693) a A	0.14469	0.14474 (0.13041 - 0.15908) a B
R_0	70.054	70.054 (50.1476 - 89.960) a A	130.86	130.860 (87.4677 - 174.253) a A
T (d)	27.9862	28.0550 (25.1280 - 30.9821) a A	33.6867	33.7137 (30.9499 - 36.4775) a A
DT (d)	4.56515	4.56415 (4.09458 - 5.03372) a A	4.79057	4.77914 (4.30695 - 5.25132) a B
λ	1.16397	1.16355 (1.14561 - 1.18149) a A	1.15568	1.15572 (1.13914 - 1.17230) a B
Parameter	SLN-HOT		SLN-COLD	
	True calculation	Jackknife estimate (95% CL)	True calculation	Jackknife estimate (95% CL)
Σm_x	174.89	-	31.03	-
r_m	0.10449	0.10285 (0.08697 - 0.11874) a B	0.11269	0.11320 (0.09525 - 0.13115) a A
R_0	49.0426	49.043 (29.3819 - 68.703) a A	22.1419	22.142 (14.5626 - 29.721) a B
T (d)	37.2543	37.7680 (29.5706 - 45.9654) a A	27.4872	27.4201 (25.4248 - 29.4154) b B
DT (d)	6.63364	6.70788 (5.71481 - 7.70096) a B	6.15103	6.08973 (5.07883 - 7.10064) a A
λ	1.11014	1.10830 (1.09065 - 1.12595) a B	1.11928	1.11982 (1.09976 - 1.13988) a A

Life table parameters were calculated using a SAS program written by Maia et al. (2000). The parameters of the Jackknife estimates on the two hosts (potato and SLN) followed by the same letter are not significantly different at P=0.05 if their 95% CL overlap. Significance for means in the same row are represented by lowercase letters and column means by uppercase letters

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Table 8 Paired comparisons between Lso-infected and Lso-free hosts – settling response of resident *B. cockerelli* adults – Field

	TRIAL 1			TRIAL 2		
	Difference	**t Value**	**Pr > |t|**	**Difference**	**t Value**	**Pr > |t|**
3 weeks-old when infected						
1 uninfected-uninfected	0.2333	0.33	0.7407	0.88	0.48	0.6389
2 uninfected-infected	0.4000	0.57	0.5713	-0.92	-0.50	0.6238
3 infected-infected	-0.03333	-0.05	0.9622	-3.52	-1.90	0.0693
5 weeks-old when infected						
1 uninfected-uninfected	-0.2000	-0.29	0.7766	5.32	2.87	**0.0083**
2 uninfected-infected	1.3667	1.96	0.0615	6.00	3.24	**0.0035**
3 infected-infected	0.7333	1.05	0.3031	-1.40	-0.76	0.4569

The 'difference' column is the actual difference in the mean number of *B.cockerelli* that settled on the two hosts in each paired comparison. A positive value indicates a greater number of *B. cockerelli* on host 1 compared to host 2 and a negative value indicate greater numbers on host 2 than host1. The P-values indicated are obtained by analyzing the difference in counts on the two hosts within a cage using repeated measures ANOVA pooled across all the six time points tested.

Table 9 Paired comparisons between Lso-infected and Lso-free hosts in the laboratory – settling response of *B. cockerelli* adults from Lso-infected and Lso-free psyllid colonies

Lso-infected adults				Lso-free adults			
Experiment	**Difference**	**t Value**	**Pr > |t|**	**Experiment**	**Difference**	**t Value**	**Pr > |t|**
1. Potato 1week after Lso infection				**11. Potato 1week after Lso infection**			
uninfected-uninfected	-2.8333	-0.91	0.3909	uninfected-uninfected	0.9583	0.30	0.7707
uninfected-infected	8.2500	2.64	**0.0298**	uninfected-infected	-5.3750	-1.68	0.1246
infected-infected	5.1667	1.65	0.1369	infected-infected	-1.0000	-0.31	0.7610
2. Potato 2weeks after Lso infection				**12. Potato 2weeks after Lso infection**			
uninfected-uninfected	- 5.1250	-1.41	0.1925	uninfected-uninfected	-0.5833	-0.14	0.8894
uninfected-infected	4.4583	1.22	0.2516	uninfected-infected	9.6667	2.37	**0.0422**
infected-infected	-4.6250	-1.27	0.2356	infected-infected	5.8333	1.43	0.1867
3. Potato 3weeks after Lso infection				**13. Potato 3weeks after Lso infection**			
uninfected-uninfected	-2.0417	-0.33	0.7457	uninfected-uninfected	-2.1667	-0.92	0.3805
uninfected-infected	15.6250	2.55	**0.0291**	uninfected-infected	-1.2500	-0.53	0.6077
infected-infected	0.2083	0.03	0.9735	infected-infected	-2.3333	-0.99	0.3466
4. Tomato 3weeks after Lso infection				**14. Tomato 3weeks after Lso infection**			
uninfected-uninfected	1.2500	0.74	0.4748	uninfected-uninfected	0.6667	0.24	0.8212
uninfected-infected	4.6250	2.73	**0.0185**	uninfected-infected	-6.5000	-2.29	0.0576
infected-infected	0.8750	0.52	0.6148	infected-infected	-5.1667	-1.82	0.1135
5. Tomato 6weeks after Lso infection				**15. Tomato 6weeks after Lso infection**			
uninfected-uninfected	-4.8333	-1.64	0.1259	uninfected-uninfected	-2.7500	-1.36	0.2101
uninfected-infected	2.5000	0.85	0.4117	uninfected-infected	0.5417	0.27	0.7954
infected-infected	1.3333	0.45	0.6583	infected-infected	0.9583	0.47	0.6479
6. Pepper 3weeks after Lso infection				**16. Pepper 3weeks after Lso infection**			
uninfected-uninfected	2.2917	1.15	0.2819	uninfected-uninfected	2.9583	0.85	0.4259
uninfected-infected	-1.0417	-0.52	0.6150	uninfected-infected	-5.5833	-1.60	0.1548
infected-infected	2.1250	1.06	0.3161	infected-infected	0.0833	0.02	0.9817

Table 9 continued. Paired comparisons between Lso-infected and Lso-free hosts in the laboratory – settling response of *B. cockerelli* adults from Lso-infected and Lso-free psyllid colonies

	Lso-infected adults				Lso-free adults		
Experiment	**Difference**	**t Value**	**Pr > |t|**	**Experiment**	**Difference**	**t Value**	**Pr > |t|**
7. Pepper 6weeks after Lso infection				**17. Pepper 6weeks after Lso infection**			
uninfected-uninfected	-4.0833	-1.16	0.2715	uninfected-uninfected	-2.4583	-1.10	0.2986
uninfected-infected	7.1250	2.03	0.0692	uninfected-infected	5.0833	2.28	**0.0487**
infected-infected	-4.1250	-1.17	0.2669	infected-infected	1.2917	0.58	0.5763
8. Eggplant 3weeks after Lso infection				**18. Eggplant 3weeks after Lso infection**			
uninfected-uninfected	1.8750	0.56	0.5862	uninfected-uninfected	3.9583	1.26	0.2512
uninfected-infected	0.2917	0.09	0.9320	uninfected-infected	-0.7917	-0.25	0.8091
infected-infected	6.4583	1.94	0.0827	infected-infected	0.3333	0.11	0.9188
9. Eggplant 6weeks after Lso infection				**19. Eggplant 6weeks after Lso infection**			
uninfected-uninfected	3.7500	1.35	0.2103	uninfected-uninfected	-1.6250	-0.68	0.5160
uninfected-infected	7.7917	2.80	**0.0206**	uninfected-infected	-2.5417	-1.06	0.3178
infected-infected	4.9167	1.77	0.1107	infected-infected	-1.5417	-0.64	0.5373
10. SLN 4weeks after Lso infection				**20. SLN 4weeks after Lso infection**			
uninfected-uninfected	-4.7083	-1.43	0.1866	uninfected-uninfected	-0.0556	-0.02	0.9836
uninfected-infected	1.0417	0.32	0.7588	uninfected-infected	-0.1667	-0.06	0.9507
infected-infected	6.0833	1.85	0.0979	infected-infected	2.5000	0.96	0.3676

The 'difference' column is the actual difference in the mean number of psyllids that settled on the two hosts in each paired comparison. A positive value indicates a greater number of psyllids on host 1 compared to host 2 and a negative value indicate greater numbers on host 2 than host1. The P-values indicated are obtained by analyzing the difference in counts on the two hosts within a cage using repeated measures ANOVA pooled across all the six time points tested.

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APPENDIX B

RESIDUAL PLOTS OF TESTS OF NORMALITY AND HOMOGENEOUS VARIANCE

Figure 1a. Adult counts in Field trial 1 (raw data before transformation)

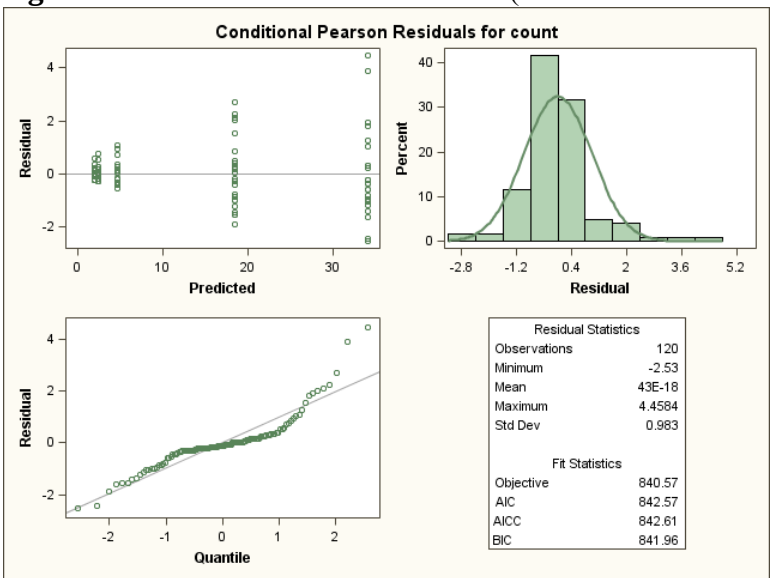

Figure 1b. Adult counts in Field trial 1 (after log transformation)

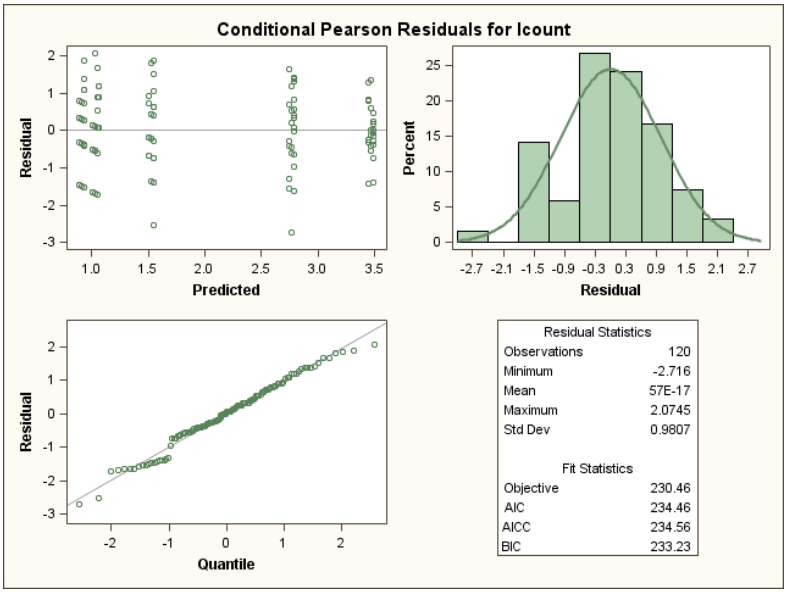

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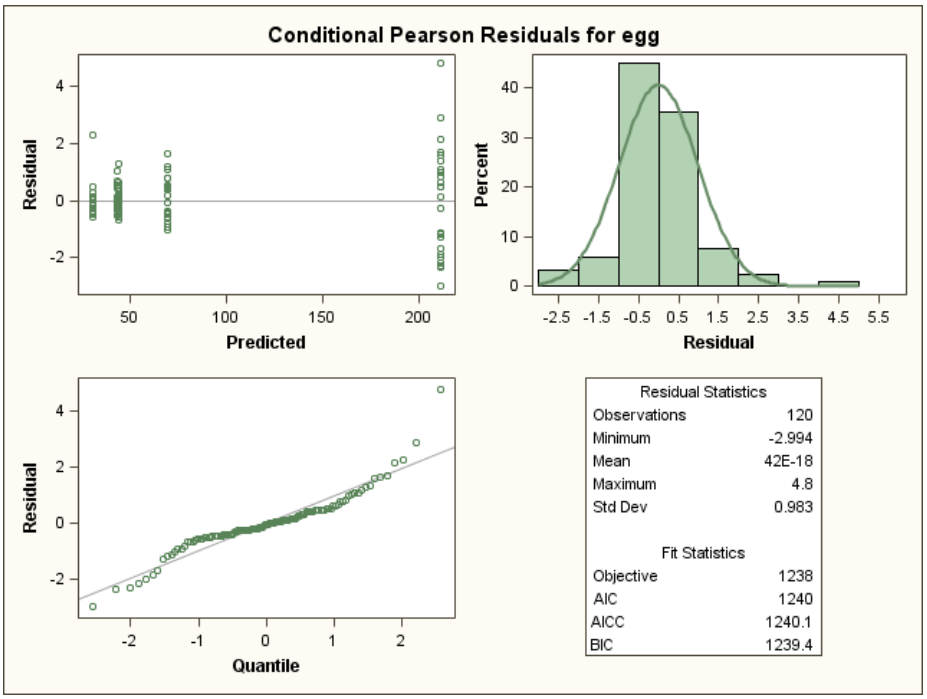
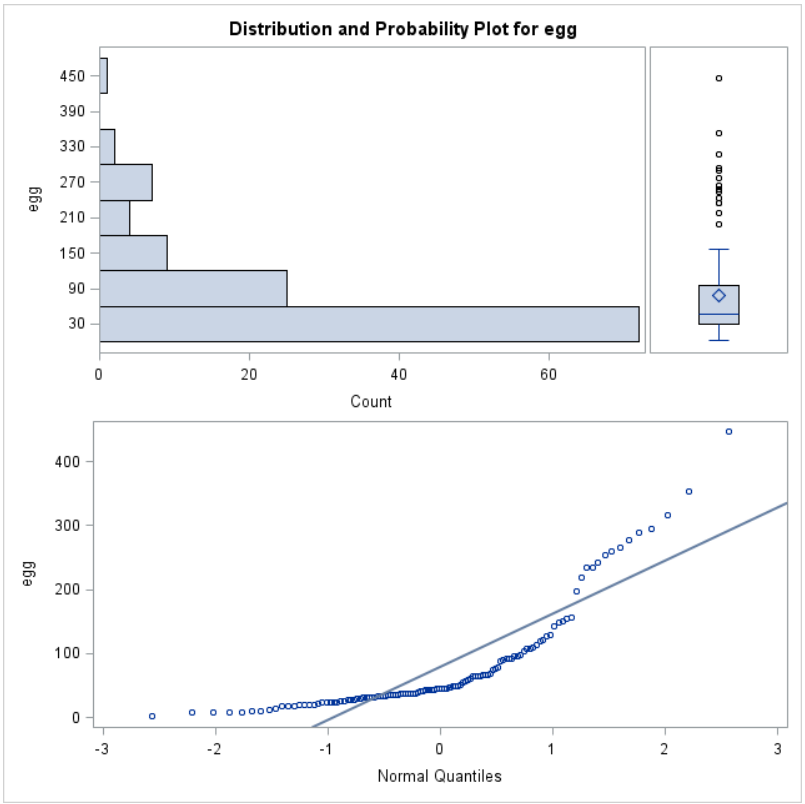
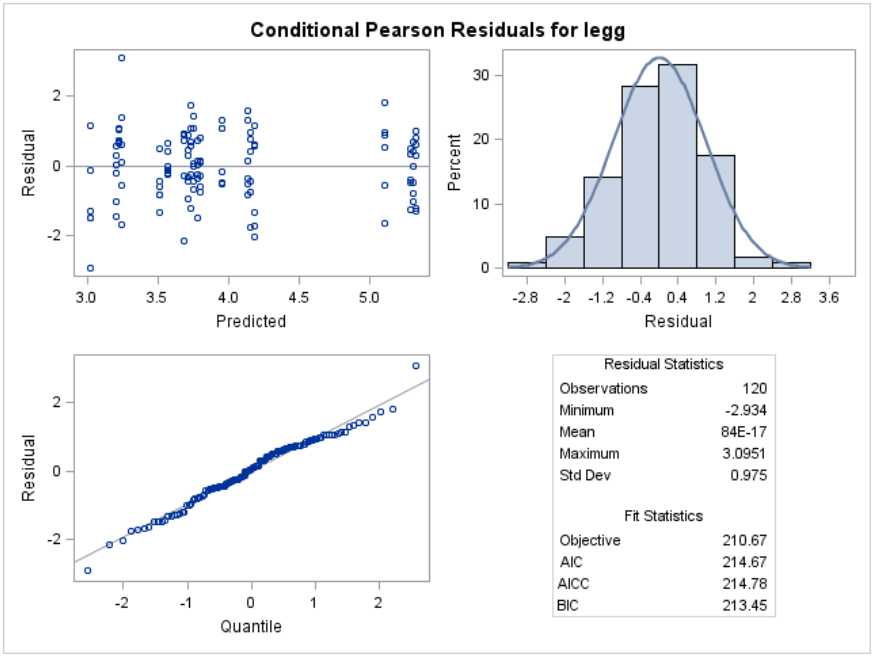
Figure 2a. Egg counts in field trial 1 (raw data before transformation)





`

Figure 2b. Egg counts in field trial 1 (after transformation)



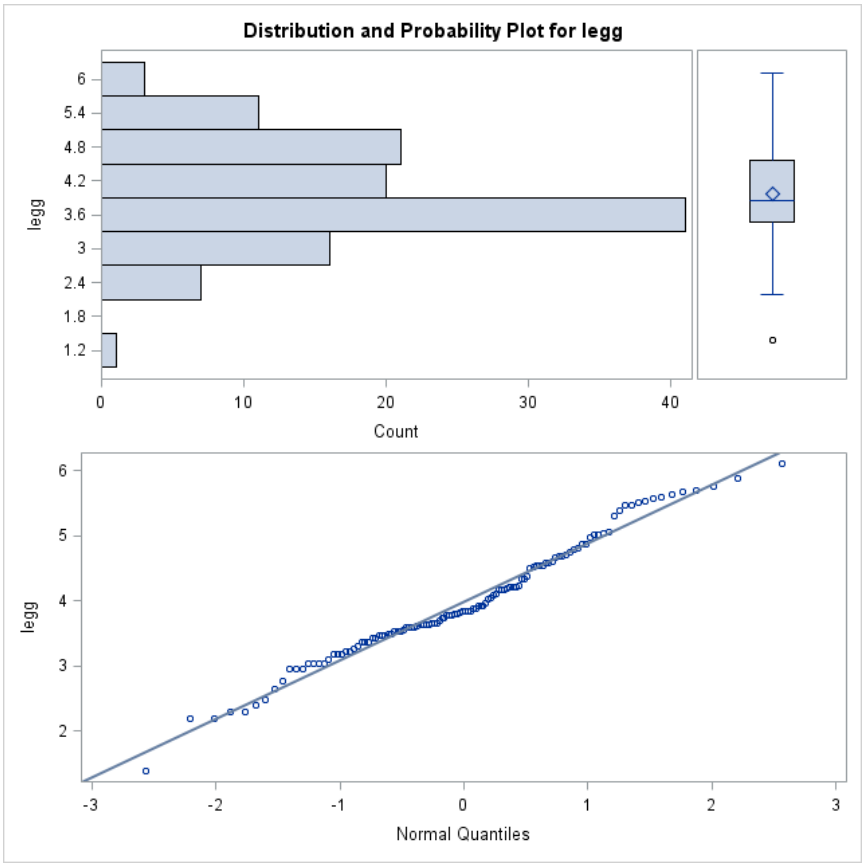


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Figure 3a. Small nymph counts in field trial 1 (raw data before transformation)

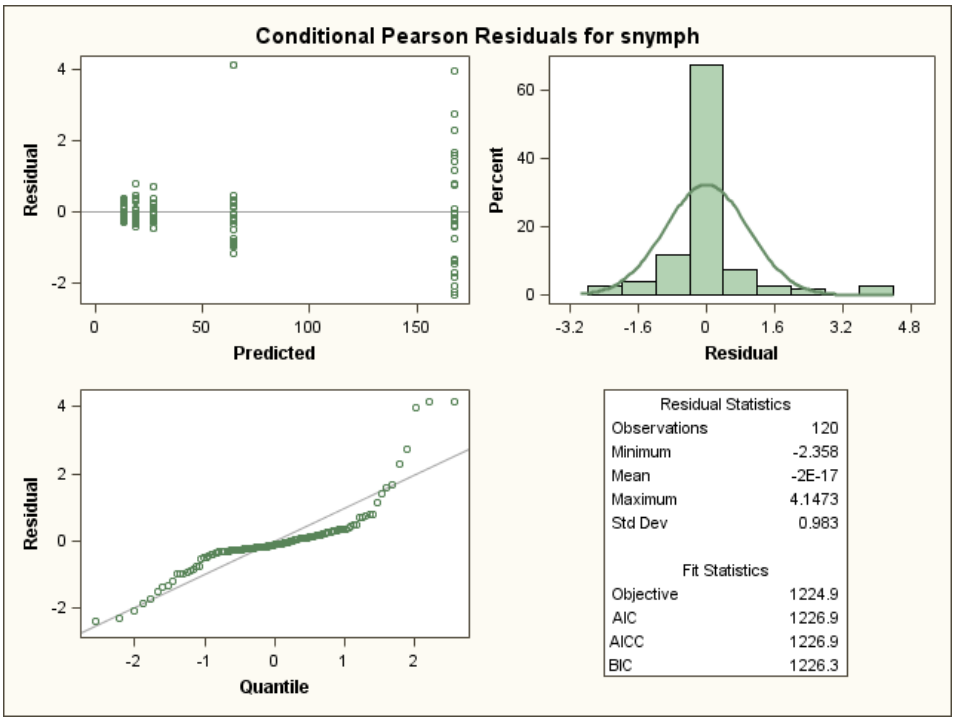

Figure 3a. Small nymph counts in field trial 1 (after log transformation)

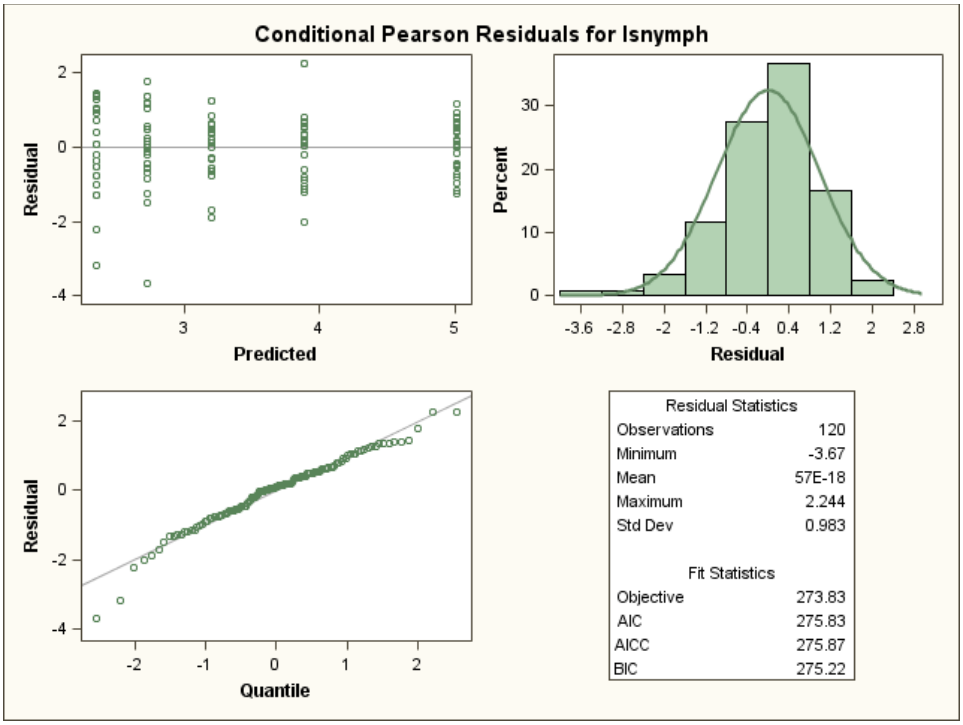

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Figure 4a. Large nymph counts in field trial 1 (raw data before transformation)

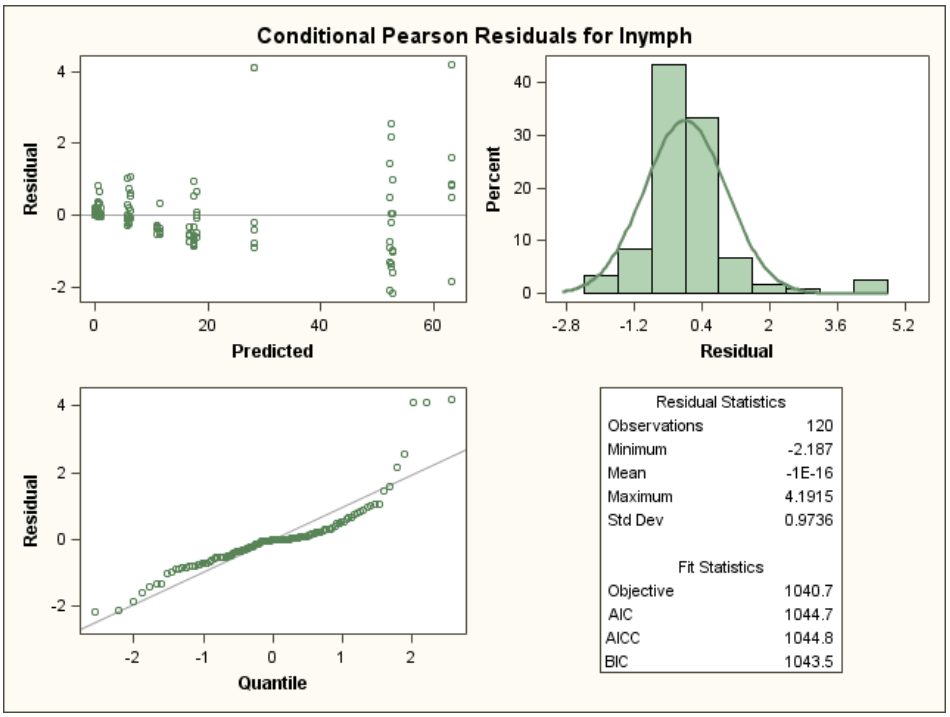
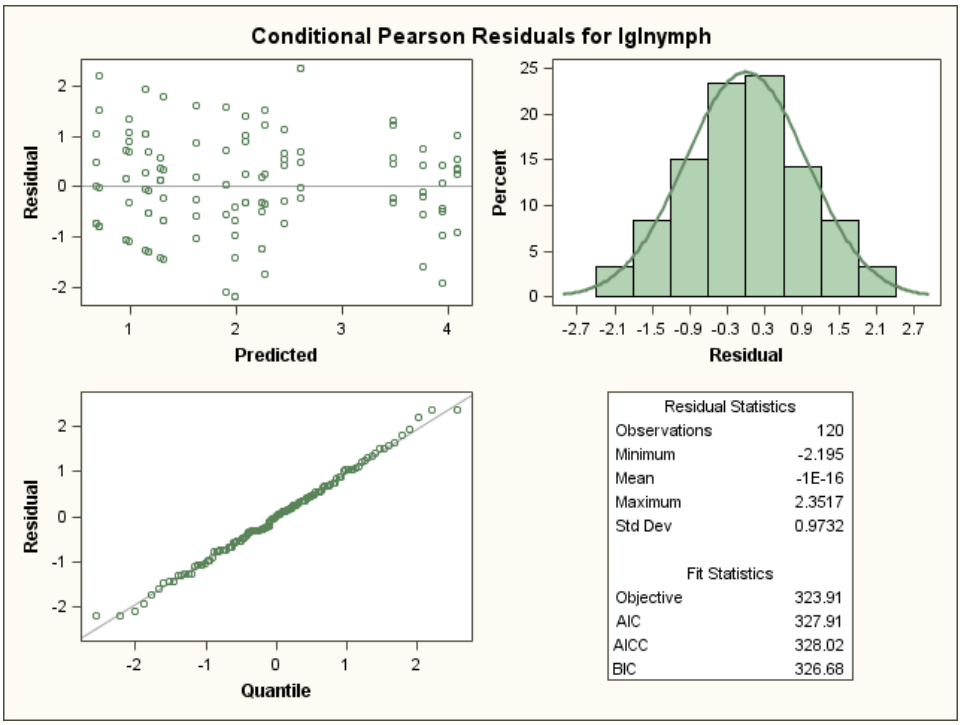


Figure 4b. Large nymph counts in field trial 1 (after log transformation)



`

Figure 5a. Adult counts in field trial 2 (raw data before transformation)

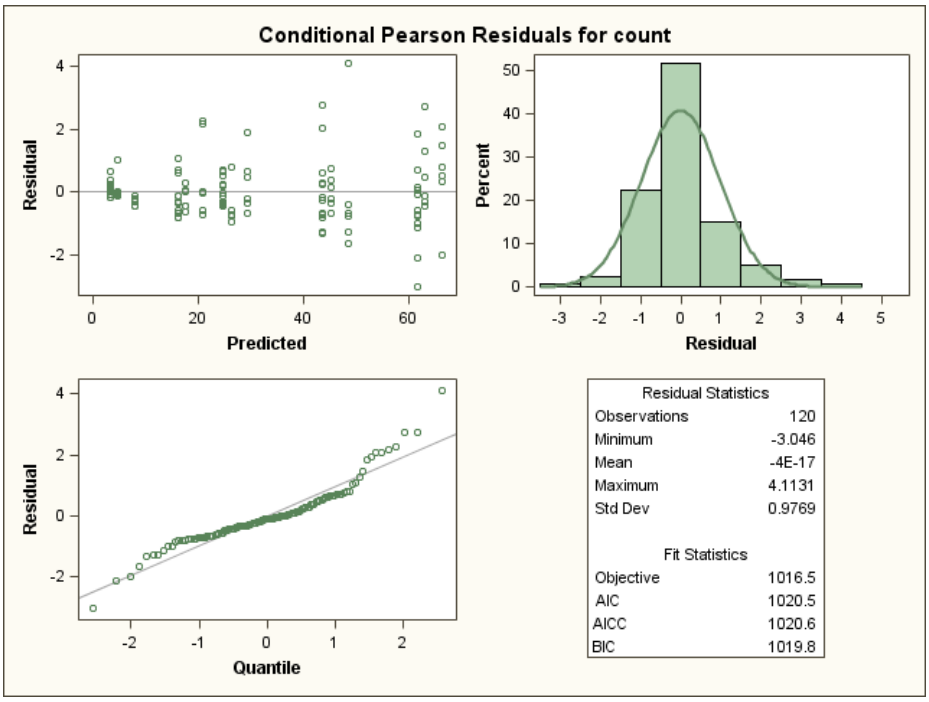


Figure 5b. Adult counts in field trial 2 (after log transformation)

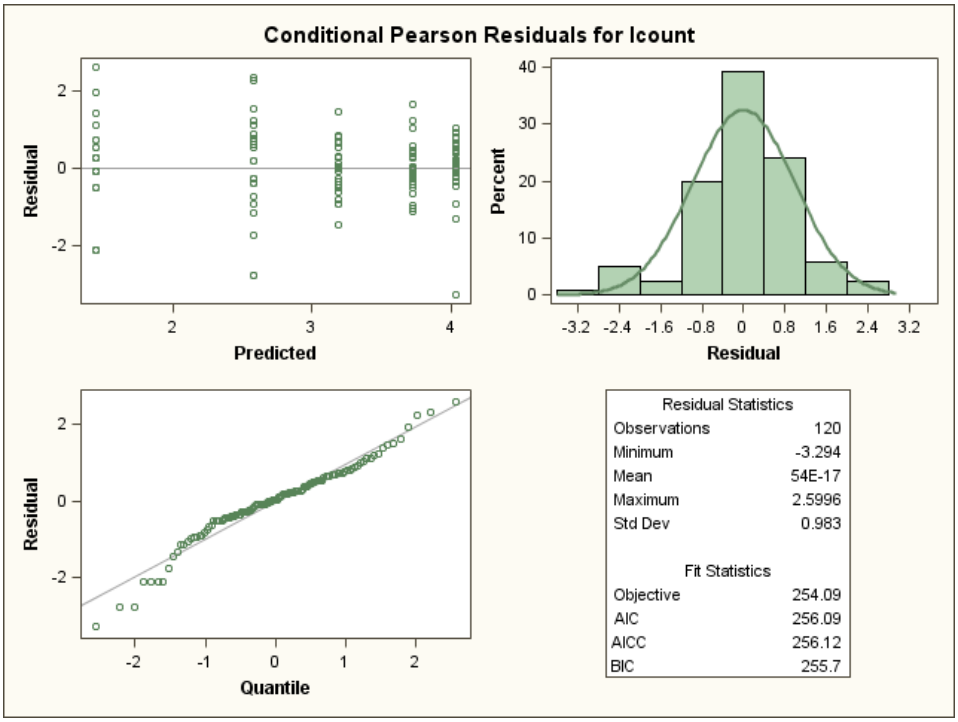


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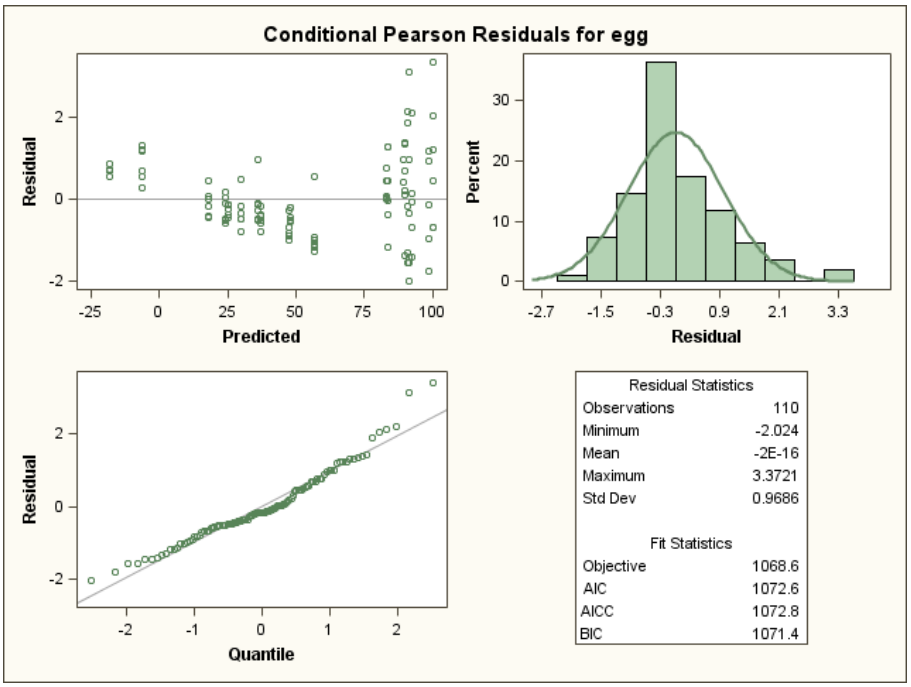
Figure 6a. Egg counts in field trial 2 (raw data before transformation)



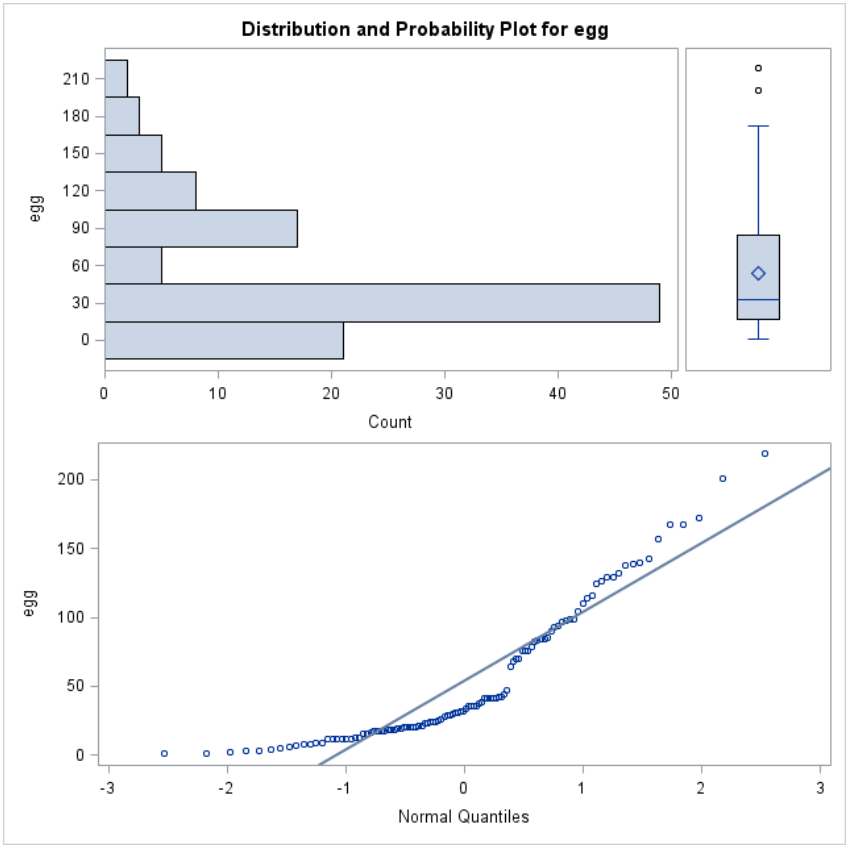


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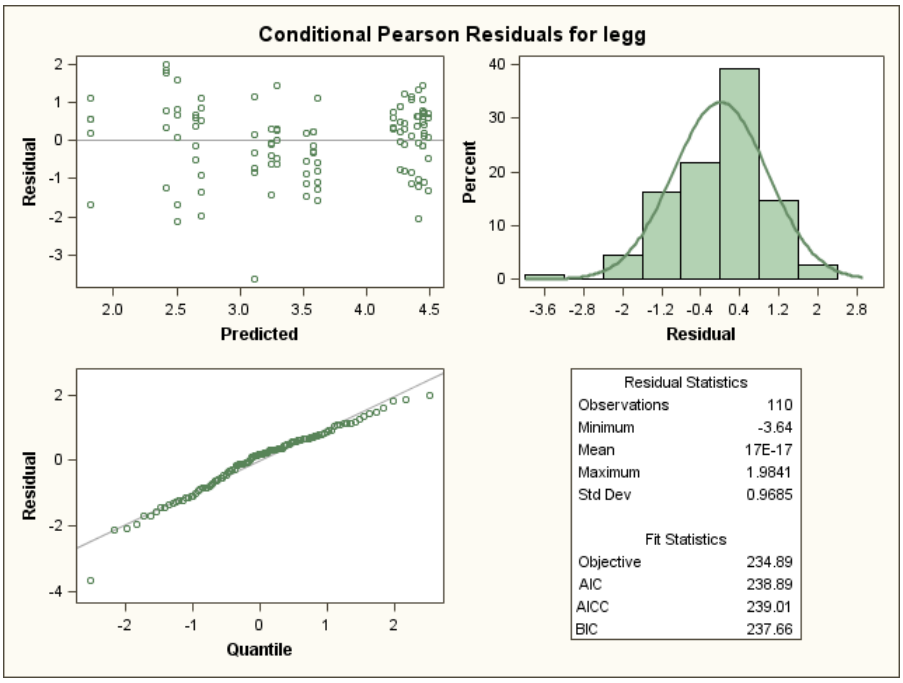
Figure 6b. Egg counts in field trial 2 (after log transformation)



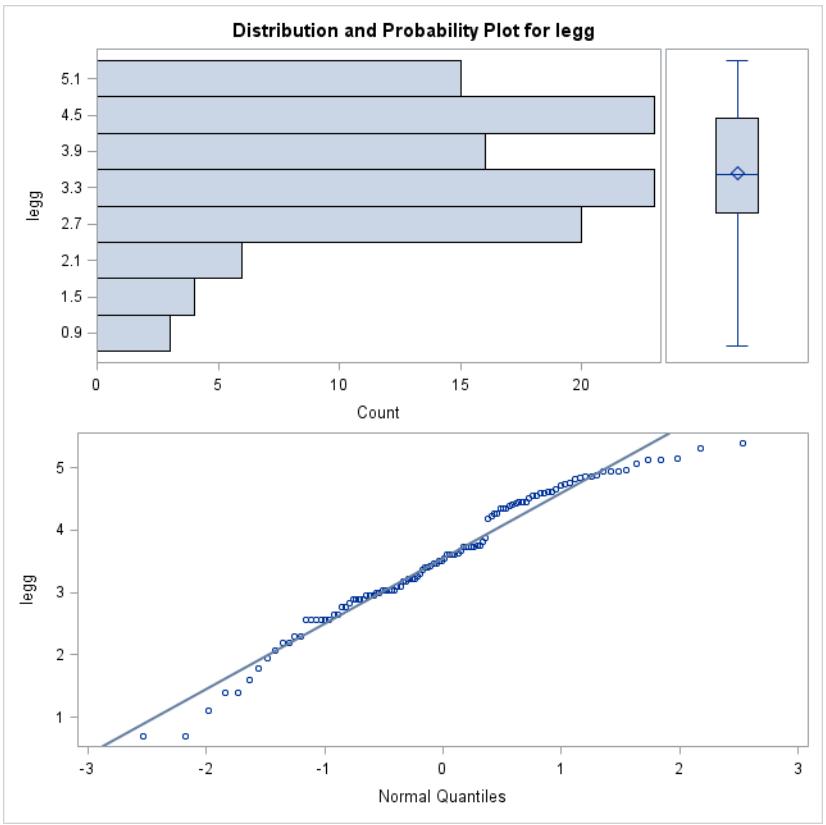


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Figure 7a. Small nymph counts in field trial 2 (raw data before transformation)

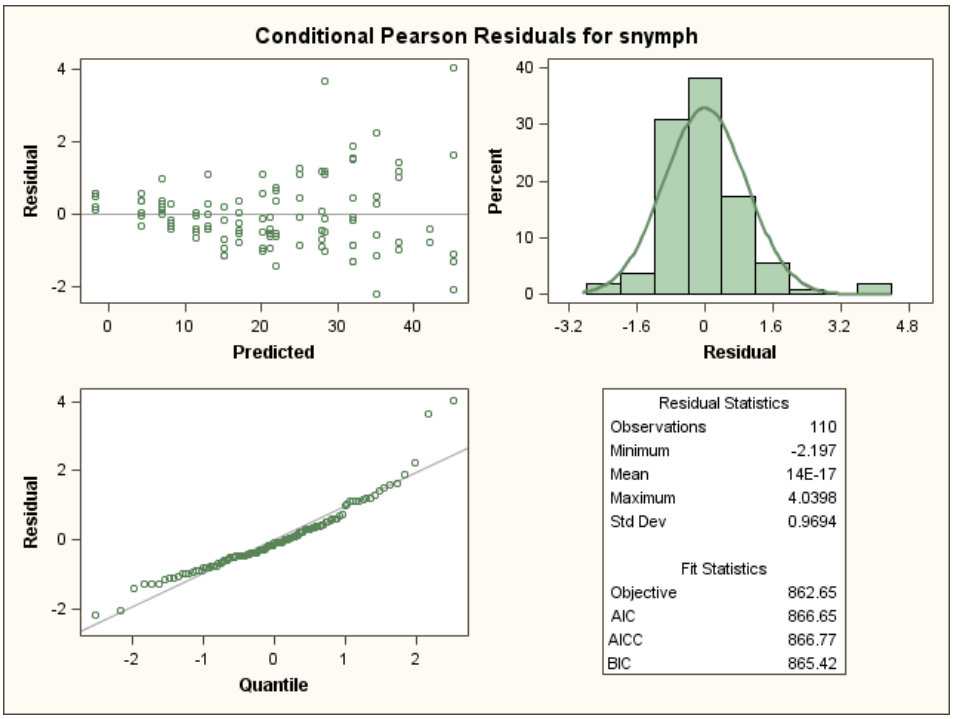

Figure 7b. Small nymph counts in field trial 2 (after log transformation)

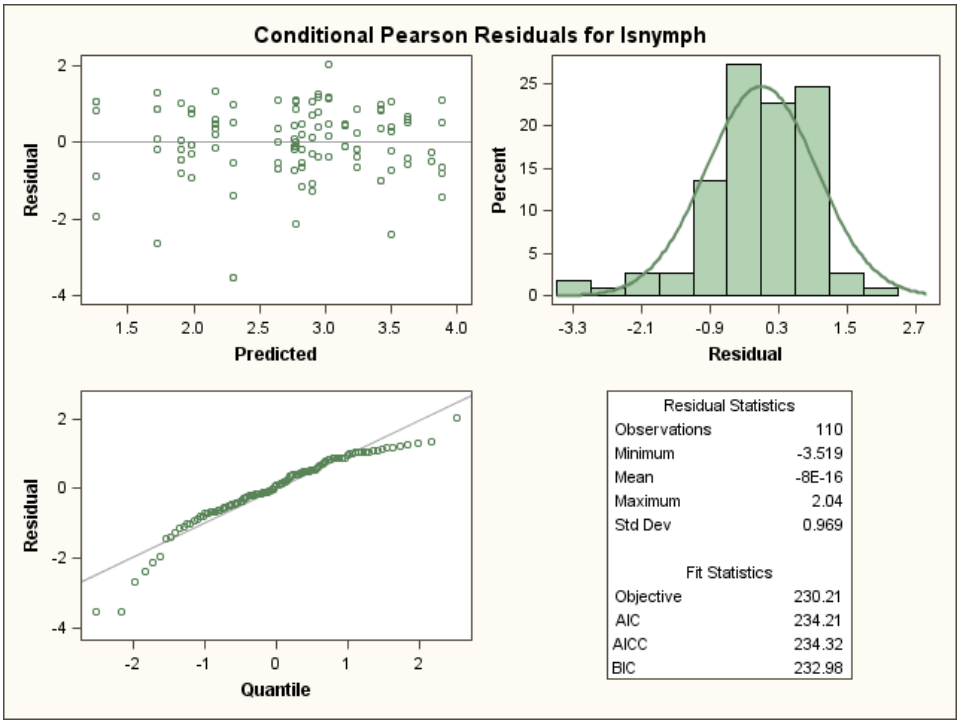

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Figure 8a. Large nymph counts in field trial 2 (raw data before transformation)

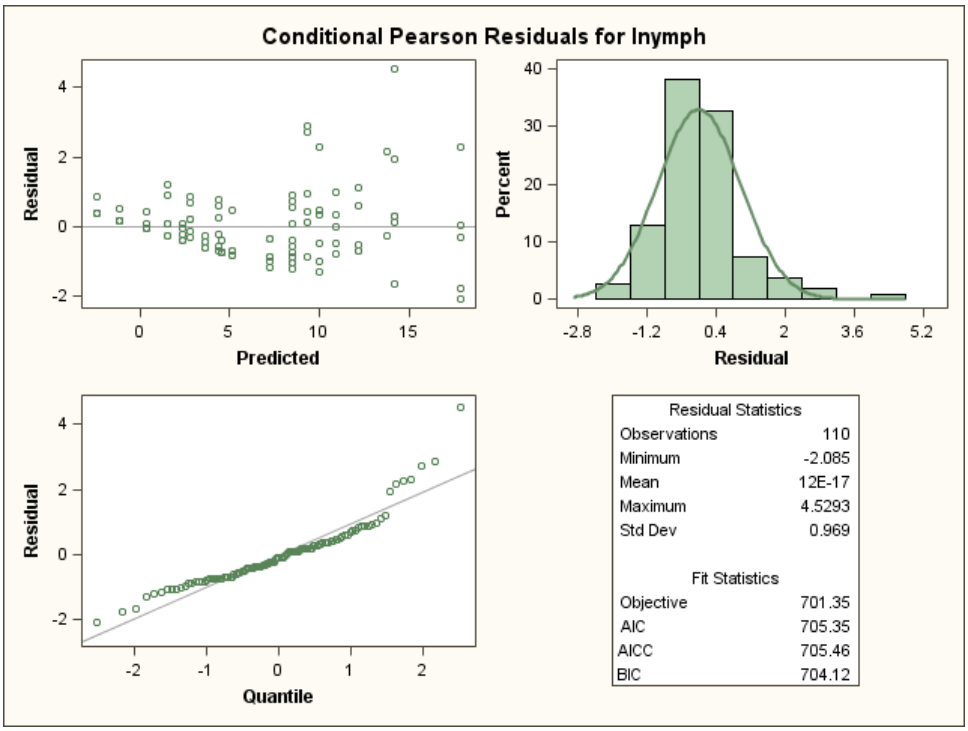

Figure 8b. Large nymph counts in field trial 2 (after log transformation)

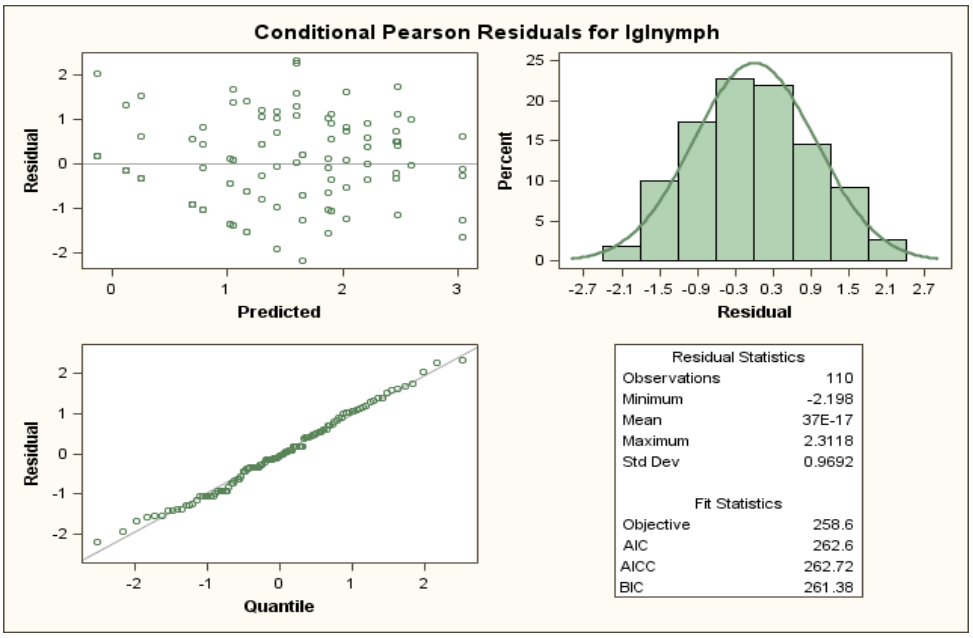

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Figure 9. Difference in adult counts on paired hosts in laboratory settling and oviposition

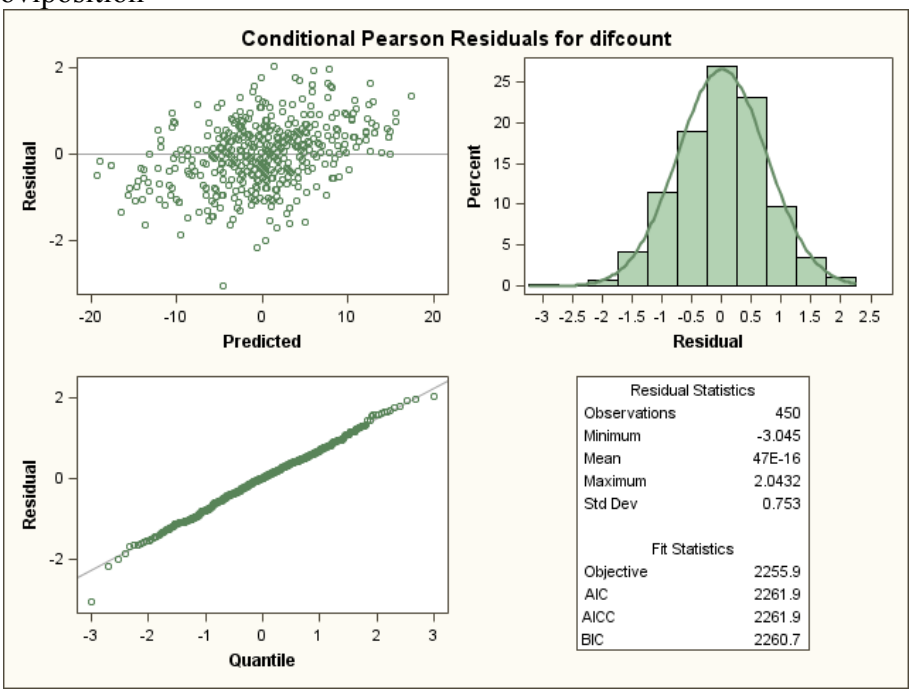

Figure 10. Differences in adult counts on paired hosts in plant size experiment

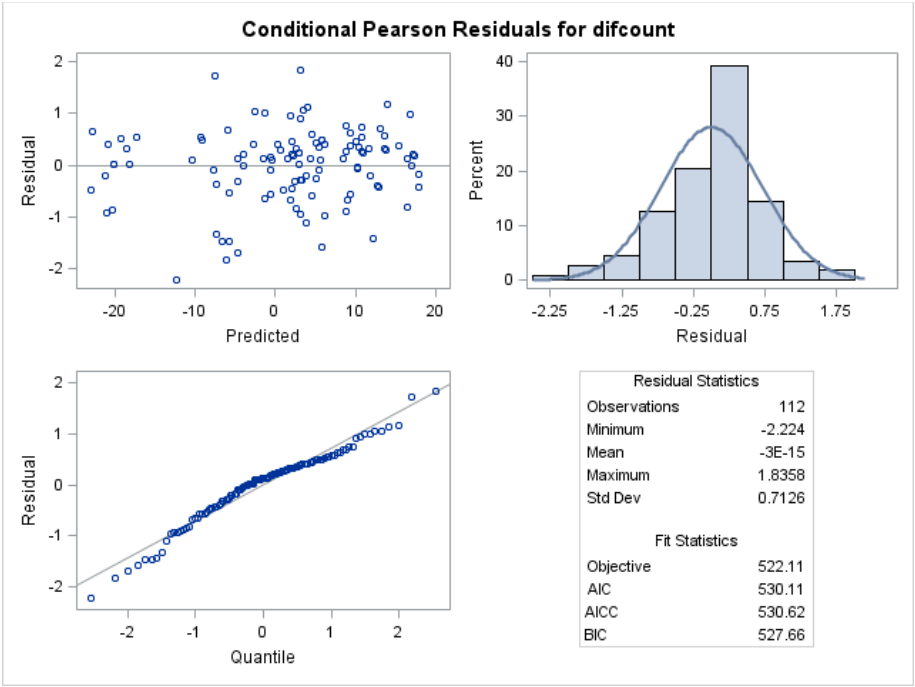

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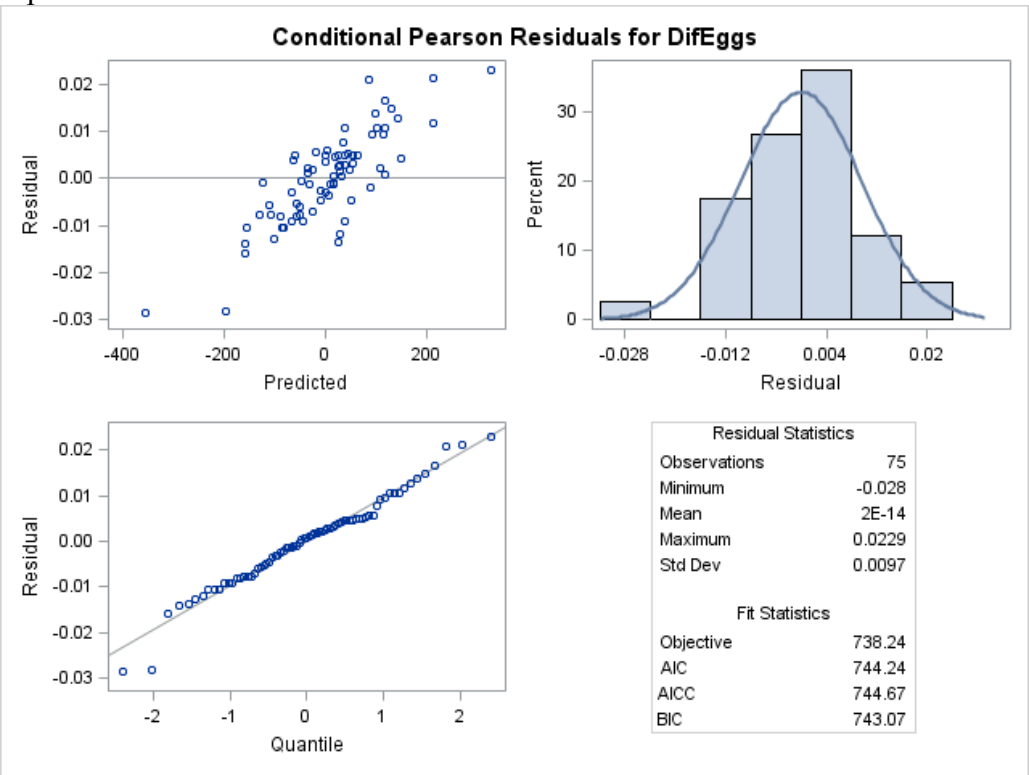
Figure 11. Differences in egg counts on paired hosts in settling and oviposition experiment



The UNIVARIATE Procedure
Moments

N	150	Sum Weights	150
Mean	0	Sum Observations	0
Std Deviation	41.8119371	Variance	1748.23809
Skewness	0	Kurtosis	0.83880105
Uncorrected SS	260487.475	Corrected SS	260487.475
Coeff Variation	.	Std Error Mean	3.41393037

Tests for Normality

Test		--Statistic---		-----p Value------
Shapiro-Wilk	W	0.989029	Pr < W	0.2899
Kolmogorov-Smirnov	D	0.053648	Pr > D	>0.1500
Cramer-von Mises	W-Sq	0.071236	Pr > W-Sq	>0.2500
Anderson-Darling	A-Sq	0.477522	Pr > A-Sq	0.2391

```
  Stem Leaf                     #             Boxplot
  12 5                          1              0
  11 1                          1              0
  10 0                          1              |
   9 44                         2              |
   7 5                          1              |
   6 03456                      5              |
   5 00118                      5              |
   4 134569                     6              |
   3 033488889                  9              |
   2 0122346688899             13             +-----+
   1 001222344555667           15             |     |
   0 1244446677778899          16             *--+--*
  -0 9988777766444421          16             |     |
  -1 766555443222100           15             |     |
  -2 9988866432210             13             +-----+
  -3 988884330                  9                |
  -4 965431                     6                |
  -5 81100                      5                |
  -6 65430                      5                |
  -9 44                         2                |
 -10 0                          1                |
 -11 1                          1               0
 -12 5                          1               0
                labeggs choice     Normal Probability Plot

                                                 125+
   |                                                *  ++
   |                                               ** ++
   |                                                ++
  75+                                             +*+
   |                                             ***
   |                                          +***
   |                                        +***
   |                                      +**
  25+                                   +***
   |                                  ***
   |                              ****
   |                          ****
   |                        ***
 -25+                   ***+
   |                 **+
   |              ***+
   |           ***+
   |         ***
 -75+      +*+
   |    ++
   |  ++ **
   |++  *
   |  *
-125+*
    +----+----+----+----+----+----+----+----+----+----+
        -2        -1         0        +1        +2
```

`

Figure 12. Difference in adult counts on infected and uninfected potato – Field trial 1

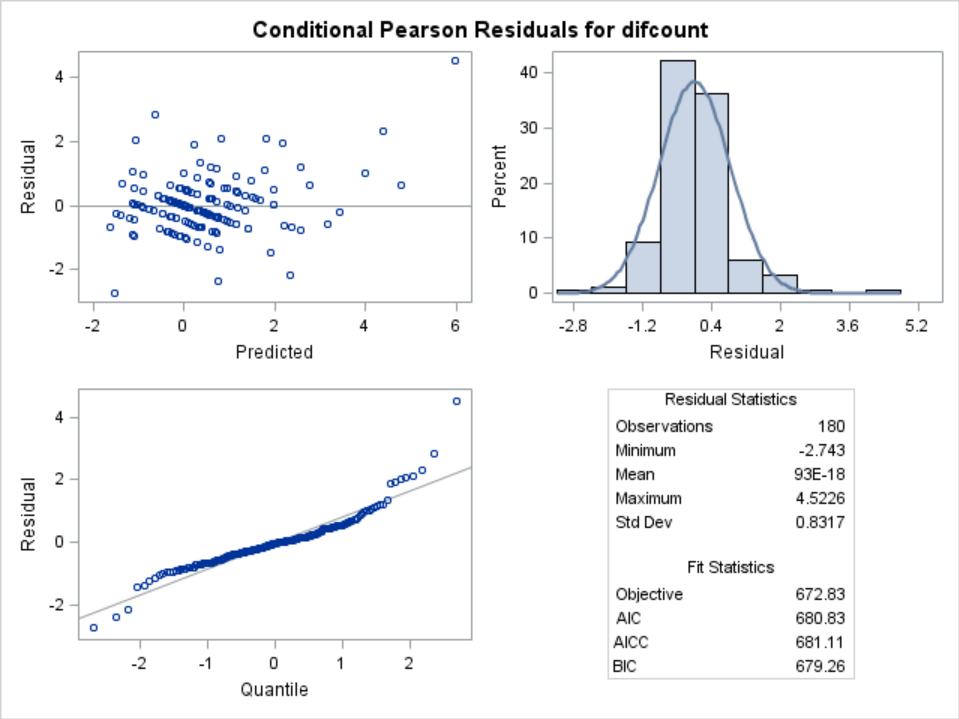
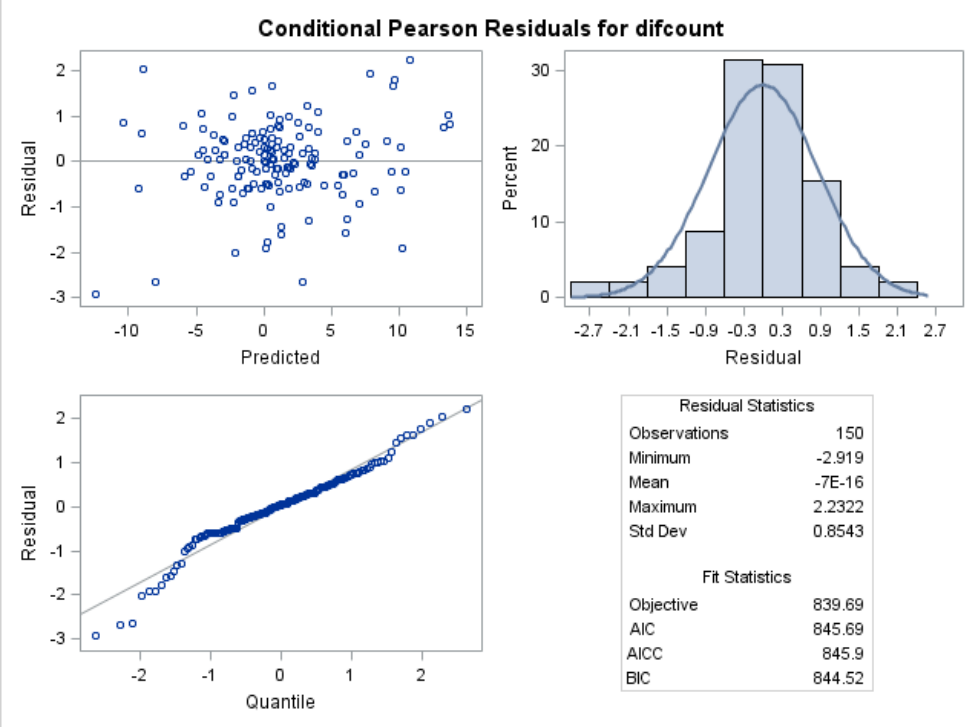


Figure 13 . Difference in adult counts on infected and uninfected potato – Field trial 2



`

Figure 14. Difference in adult counts on potato- one week after Lso-infection – Lso-free psyllids

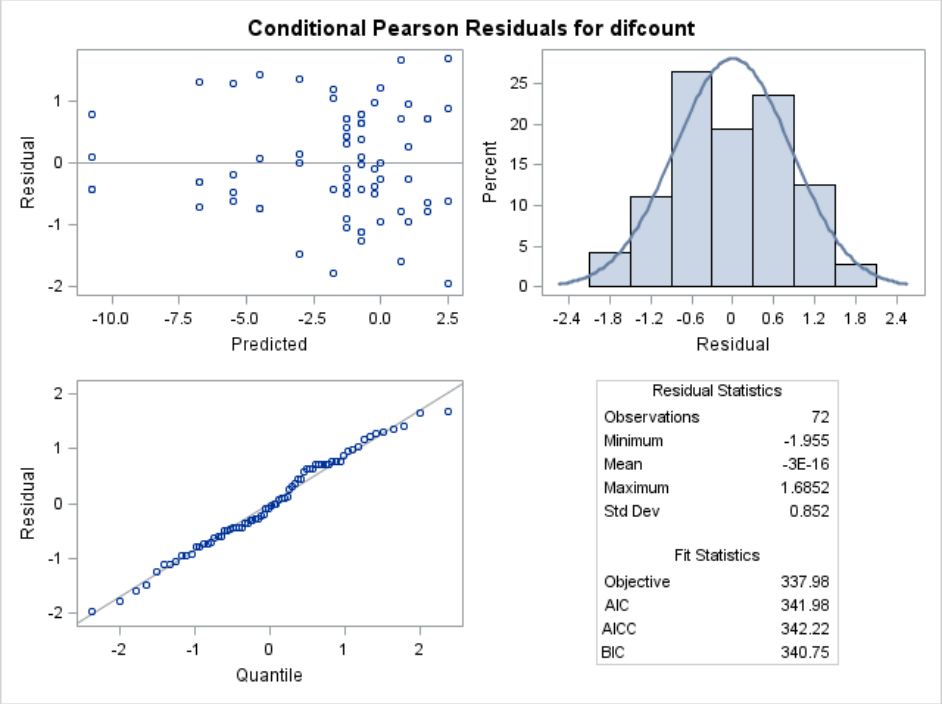


Figure 15. Difference in adult count on potato- two weeks after Lso-infection – Lso-free psyllids

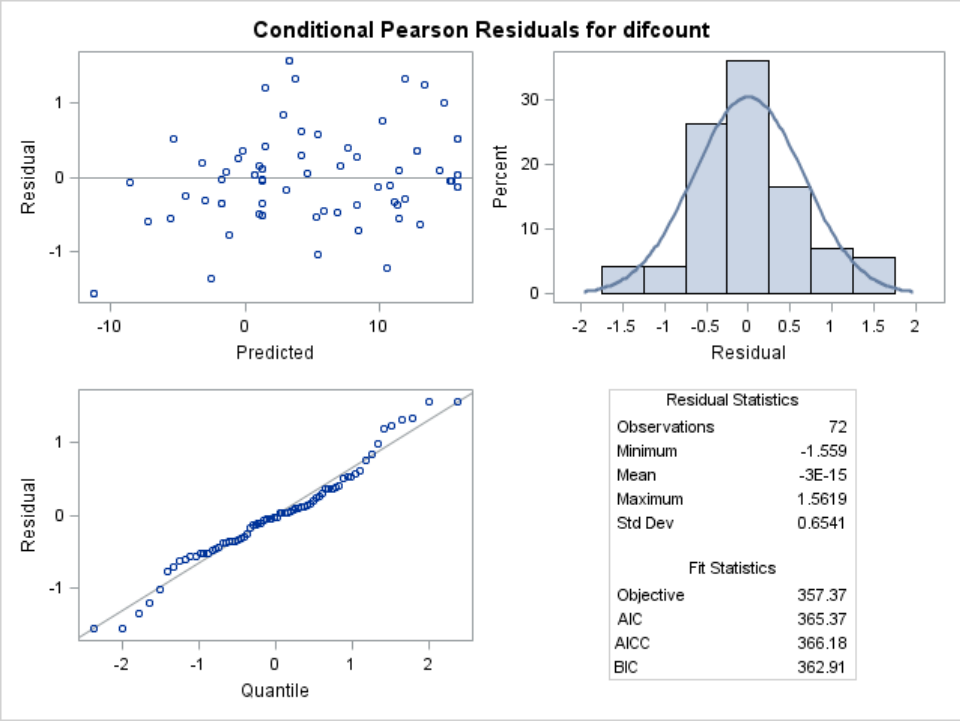


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Figure 16. Difference in adult counts on potato- three weeks after Lso-infection – Lso-free psyllids

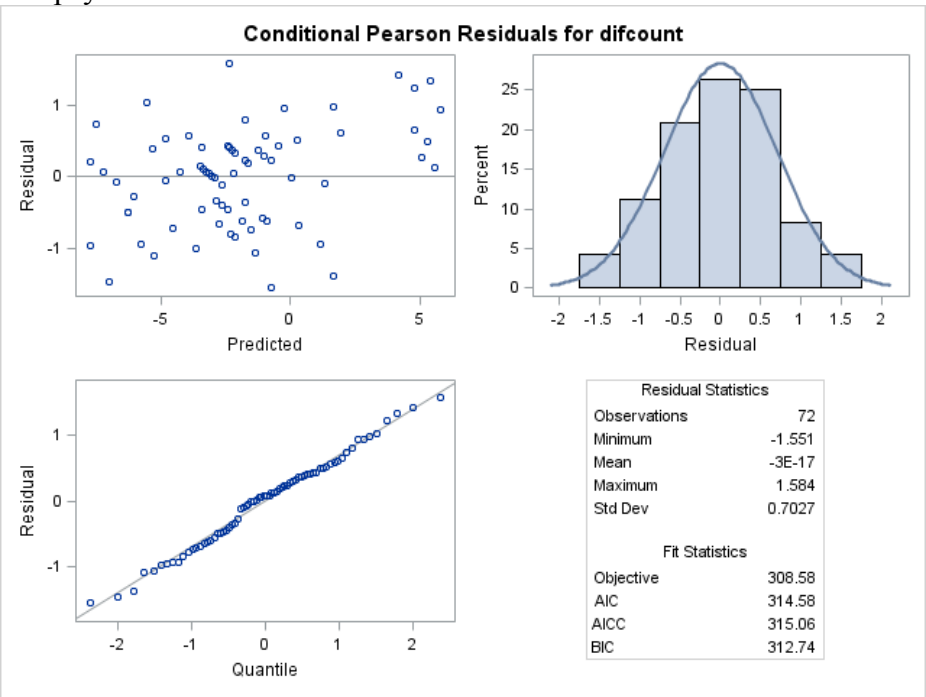

Figure 17. Difference in adult counts on tomato- three weeks after Lso-infection – Lso-free psyllids

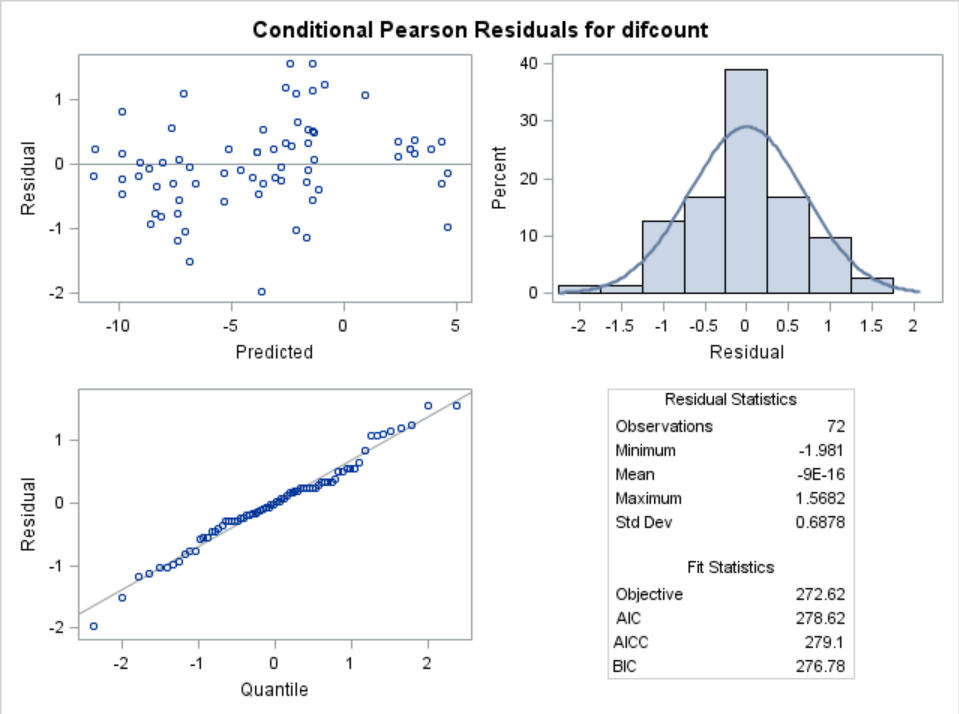

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Figure 18. Difference in adult counts on tomato- six weeks after Lso-infection – Lso-free psyllids

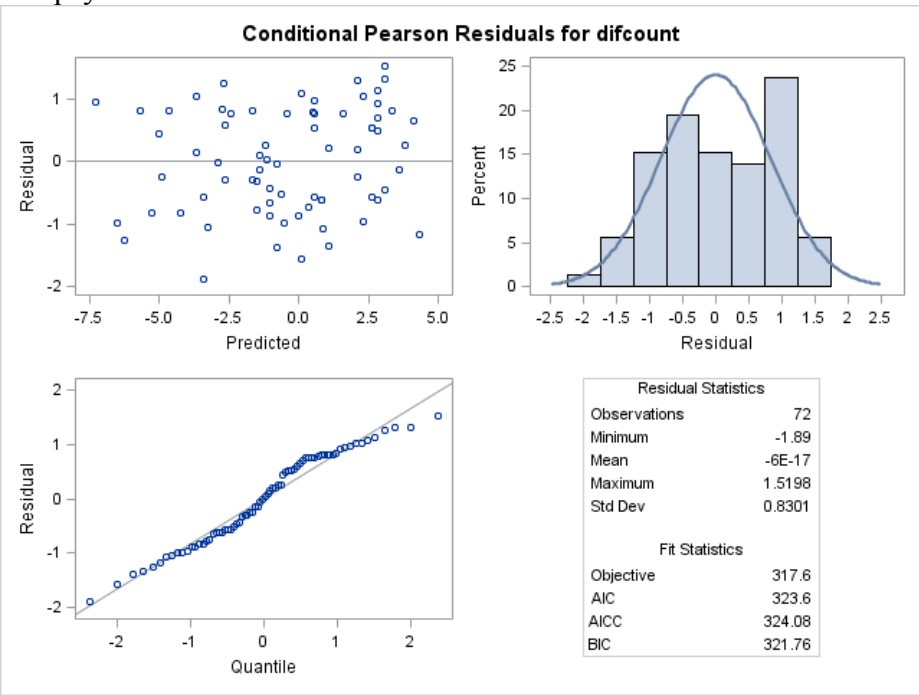
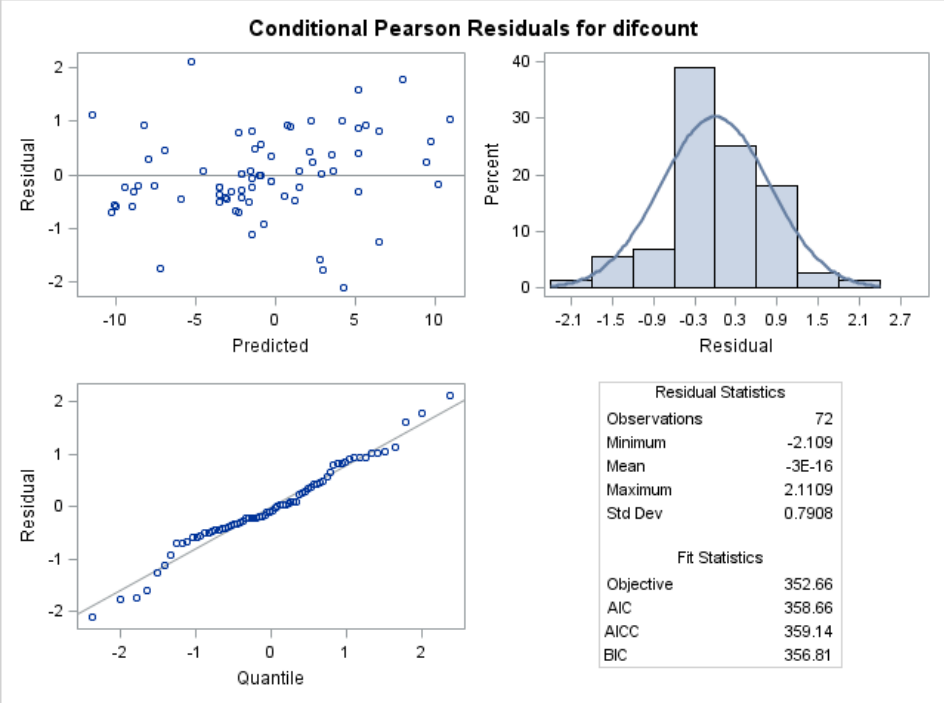


Figure 19. Difference in adult counts on pepper- three weeks after Lso-infection – Lso-free psyllids



`

Figure 20. Difference in adult counts on pepper- six weeks after Lso-infection – Lso-free psyllids

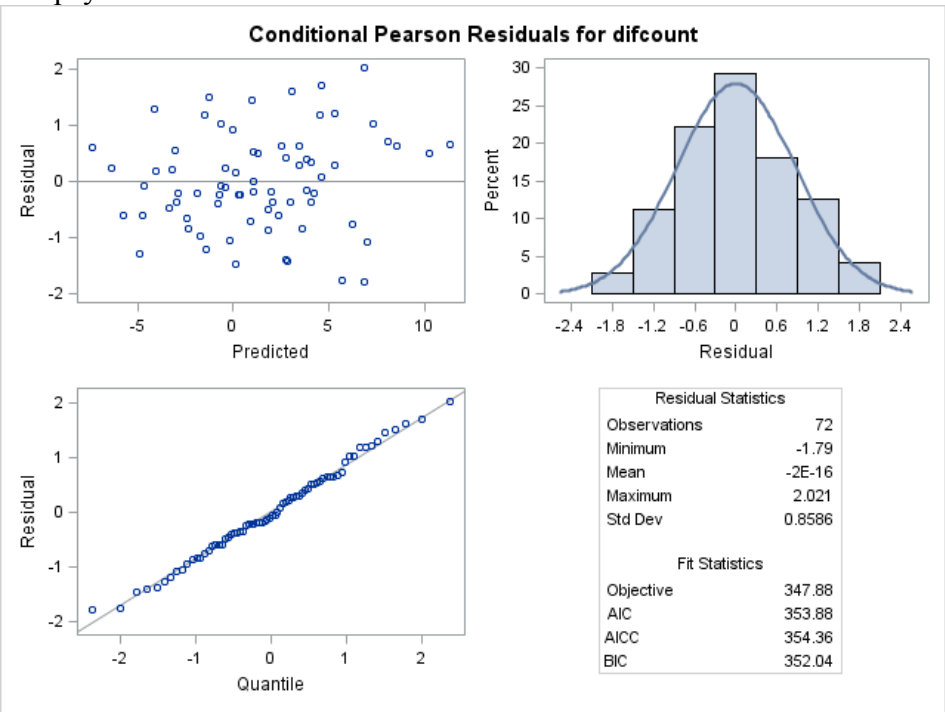


Figure 21. Difference in adult counts on eggplant- three weeks after Lso-infection – Lso-free psyllids

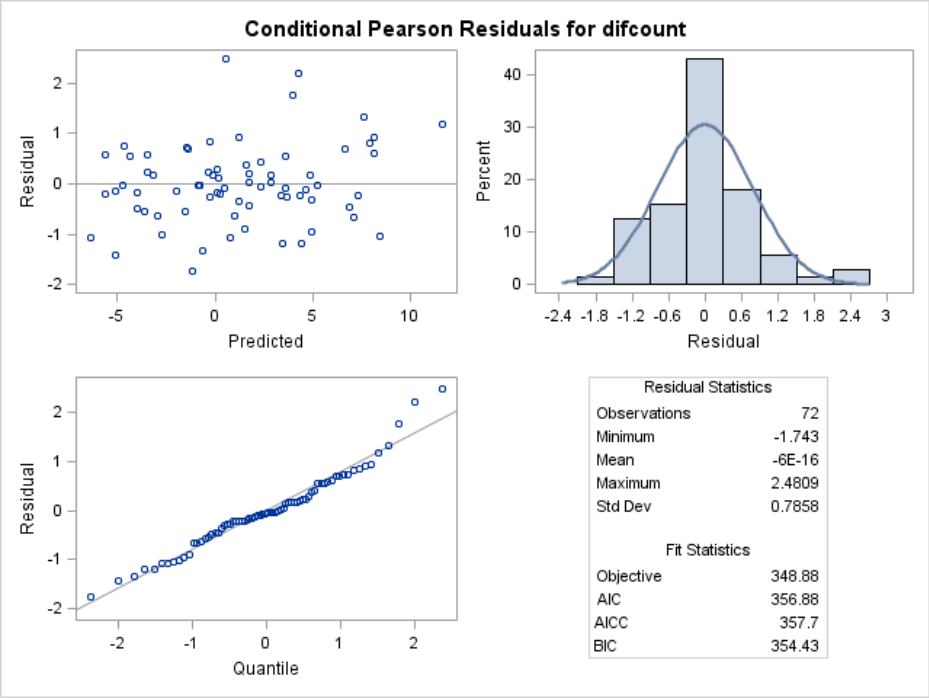


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Figure 22. Difference in adult counts on eggplant- six weeks after Lso-infection – Lso-free psyllids

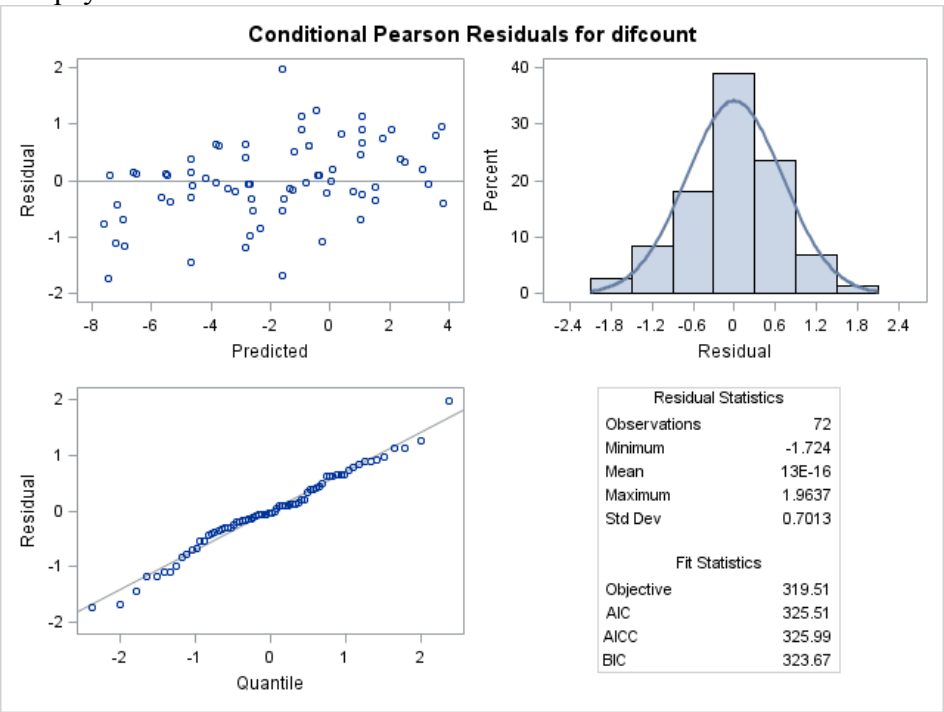
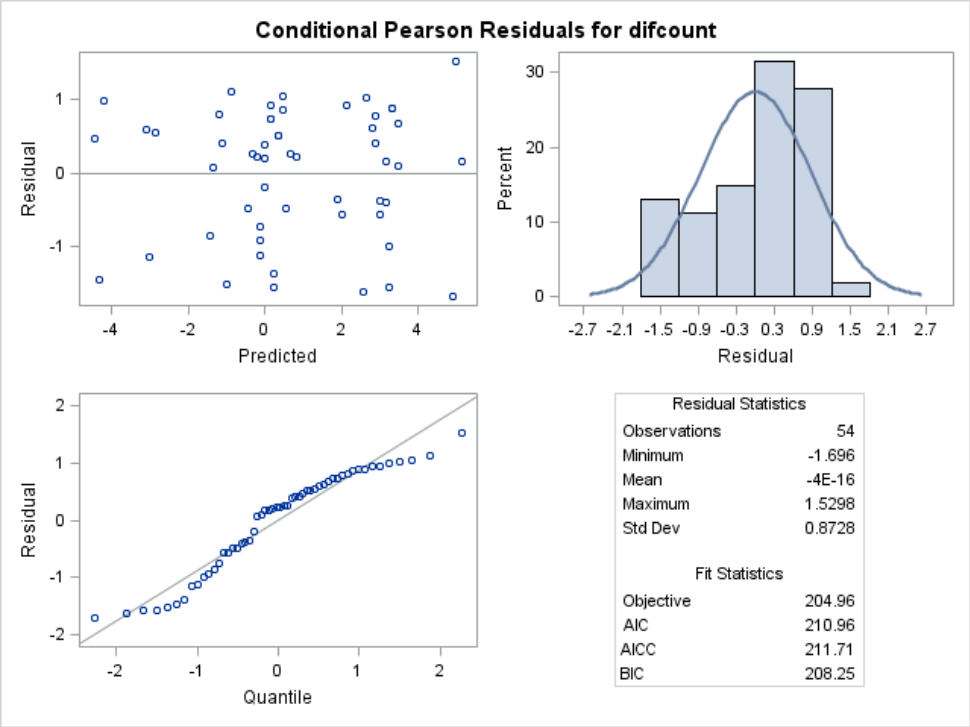


Figure 23. Difference in adult counts on SLN- four weeks after Lso-infection – Lso-free psyllids



`

APPENDIX C

SAS CODES

1. Field settling experiment

```
ODS HTML; ODS GRAPHICS ON;
options nocenter nodate ls=90 ps=60;
data exper1;
infile 'u:\consult\janita_dataV3.csv' dlm=',';
   input rep $ pair $ host $ plant $ @;
   do date=1 to 8;
   input count @;output; end;
 data lexper1;set exper1;
lcount = log(count+1);
    run;
proc print;
run;
proc mixed data=lexper1 order=data ;
   class rep pair plant host date;
   model lcount = host date host*date/residual ddfm=sat;
   random rep pair(rep);
   repeated date/subject=plant type=AR(1);
   lsmeans host date /adjust=tukey;
run;
ODS GRAPHICS OFF; ODS HTML CLOSE;
```

2. Field egg data

```
ODS HTML; ODS GRAPHICS ON;
options nocenter nodate ls=90 ps=60;
data egg1;
infile 'C:\JENITAC\AAAAA\SAS\field1eggs.csv' dlm=',' firstobs=3;
   input rep $ pair $ host $ plant $ egg1-egg3;
    data legg1;set egg1;
      egg = egg1+egg2+egg3;
legg = log(egg+1);
    run;
proc print;
run;
proc mixed data=legg1 order=data ;
   class rep  plant host;
   model egg = host /residual ddfm=sat;
   random rep ;
```

`

```
   lsmeans host  /adjust=tukey;
run;
proc univariate  plot normal; VAR egg;
proc mixed data=legg1 order=data ;
   class rep  plant host;
   model legg = host /residual ddfm=sat;
   random rep ;
   lsmeans host  /adjust=tukey;
run;
proc univariate  plot normal; VAR legg;
proc sort;
by host;
proc means mean stderr;
by host;
VAR egg;
run;
ODS GRAPHICS OFF; ODS HTML CLOSE;
```

3. Analysis of laboratory settling data

```
ODS HTML; ODS GRAPHICS ON;
options nocenter nodate ls=90 ps=60;
data experlab;
infile 'u:\consult\janita_dataExp2V2.csv' dlm=',';
   input rep $ pair $ host $ plant $ Total eggs @;
   do date=1 to 6;
   input count @;output; end;
 data lexper2;set exper2;
lcount = log(count+1);
    run;
proc print;
run;
proc mixed data=lexper1 order=data ;
   class rep pair plant host date;
   model lcount = host date host*date/residual ddfm=sat;
   random rep pair(rep);
   repeated date/subject=plant type=AR(1);
   lsmeans host date /adjust=tukey;
run;
ODS GRAPHICS OFF; ODS HTML CLOSE;
```

4. Analysis of laboratory egg data

```
ODS HTML; ODS GRAPHICS ON;
options nocenter nodate ls=90 ps=60;
data labdifegg;
```

`

```
infile 'C:\JENITAC\AAAAA\SAS\LabDiffEggs.csv' dlm=','
firstobs=4;
   input rep $ hostpair $  cage $ DifEggs @;

proc print data=labdifegg;
run;
proc mixed data=labdifegg order=data ;
   class rep cage hostpair;
   model DifEggs = hostpair /residual ddfm=sat;
   random rep cage(rep);
   lsmeans hostpair /adjust=tukey;
run;
ODS GRAPHICS OFF; ODS HTML CLOSE;
```

5. Analysis of pre-adaptation experiment

```
ODS HTML; ODS GRAPHICS ON;
options nocenter nodate ls=90 ps=60;
data preadapt;
infile 'C:\JENITAC\AAAAA\SAS\preadapt difcount.csv' dlm=','
firstobs=3;
   input rep $ pair $  cage $ @;
   do count=1 to 6;
   input difcount @;output; end;

proc print data=preadapt;
run;
proc mixed data=preadapt order=data ;
   class rep cage pair count;
   model difcount = count pair count*pair/residual ddfm=sat;
   random rep cage(rep pair);
   repeated count/subject=cage(rep pair) type=AR(1);
   lsmeans count pair count*pair/adjust=tukey;
run;
ODS GRAPHICS OFF; ODS HTML CLOSE;
```

6. Analysis of field plot density experiment

```
ODS HTML; ODS GRAPHICS ON;
options nocenter nodate ls=90 ps=60;
data zookasplitperplant;
infile 'C:\JENITAC\AAAAA\SAS\insectzookasplit perplant.csv'
dlm=','  firstobs=3;
   input rep $ var $ group $ patchsize $  @;
   do date=1 to 7;
   input count @;output; end;
```

`

```
proc print data=zookasplitperplant;
run;
proc mixed data=zookasplitperplant order=data ;
   class rep patchsize var date group;
   model count = patchsize var var*patchsize date date*var
date*patchsize var*date*patchsize/residual ddfm=sat;
   random rep rep*patchsize / solution;
   repeated date/subject=group(rep) type=AR(1);
   lsmeans patchsize | var/adjust=tukey;
run;
ODS GRAPHICS OFF; ODS HTML CLOSE;
```

7. Analysis of lso-uninfected and infected field data

```
ODS HTML; ODS GRAPHICS ON;
options nocenter nodate ls=90 ps=60;
data field1uninfec;
infile 'C:\JENITAC\AAAAA\SAS\field1uninfec infected.csv' dlm=','
firstobs=3;
   input rep $ pair $  cage $  age $ @;
   do date=1 to 6;
   input difcount @;output; end;

proc print data=field1uninfec;
run;
proc mixed data=field1uninfec order=data ;
   class rep cage pair date;
   model difcount = pair date pair*date/residual ddfm=sat;
   random rep cage(rep);
   repeated date/subject=cage(rep) type=AR(1);
   lsmeans pair date pair*date/adjust=tukey;
run;
ODS GRAPHICS OFF; ODS HTML CLOSE;
```

8. Analysis of lso-uninfected and infected-laboratory data

```
ODS HTML; ODS GRAPHICS ON;
options nocenter nodate ls=90 ps=60;
data eggcold3week;
infile 'C:\JENITAC\AAAAA\SAS\uninf eggplantcold 3weeksdif.csv'
dlm=','  firstobs=3;
   input rep $ pair $  cage $ @;
   do time=1 to 6;
   input difcount @;output; end;
 proc print data=eggcold6weeks;
```

`

```
run;
proc mixed data=eggcold3week order=data ;
   class rep cage pair time;
   model difcount = pair time pair*time/residual ddfm=sat;
   random rep cage(rep);
   repeated time/subject=cage(rep) type=AR(1);
   lsmeans pair time pair*time/adjust=tukey;
run;
ODS GRAPHICS OFF; ODS HTML CLOSE;
```

9. Analysis of egg and nymphal development

```
ODS HTML; ODS GRAPHICS ON;
options nocenter nodate ls=90 ps=60;
data devdays;
infile 'u:\consult\JenitaT\lifetable-combined-devdays.csv'
dlm=','  firstobs=3;
input cage $ insect $ egg S1 S2 S3 S4 S5 host $;

proc print;
run;
proc means mean stderr;
class host;
var egg S1-S5 ;
run;
proc means mean stderr;
var egg S1-S5 ;
run;
proc mixed data=devdays order=data ;
   class cage host insect;
   model egg  = host/residual ddfm=sat;
   random cage;
   lsmeans host/adjust=tukey;
run;
proc mixed data=devdays order=data ;
   class cage host insect;
   model S1  = host/residual ddfm=sat;
   random cage;
   lsmeans host/adjust=tukey;
run;
proc mixed data=devdays order=data ;
   class cage host insect;
   model S2  = host/residual ddfm=sat;
   random cage;
   lsmeans host/adjust=tukey;
run;
proc mixed data=devdays order=data ;
   class cage host insect;
```

`

```
   model S3  = host/residual ddfm=sat;
   random cage;
   lsmeans host/adjust=tukey;
run;
proc mixed data=devdays order=data ;
   class cage host insect;
   model S4  = host/residual ddfm=sat;
   random cage;
   lsmeans host/adjust=tukey;
run;

proc mixed data=devdays order=data ;
   class cage host insect;
   model S5  = host/residual ddfm=sat;
   random cage;
   lsmeans host/adjust=tukey;
run;
proc glm data=devdays order=data ;
   class cage host insect;
   model  S1-S5  = cage host/nouni;
   random cage;
   manova h=host;
run;
```

10. Analysis of longevity, oviposition, pre-oviposition and eggs laid

```
ODS HTML; ODS GRAPHICS ON;
options nocenter nodate ls=90 ps=60;
data longevity;
infile 'C:\JENITAC\AAAAA\SAS\lifehistory longevity.csv' dlm=','
firstobs=2;
   input host $ npsyllid $ long preovi ovi neggs;

proc print;
run;
proc means mean stderr;
class host;
var long preovi ovi neggs;
run;
proc mixed data=longevity order=data ;
   class host npsyllid;
   model long = host  /solution residual ddfm=sat;
   random npsyllid;
   lsmeans host /adjust=tukey;
run;
proc mixed data=longevity order=data ;
   class host npsyllid;
```

`

```
   model preovi = host  /solution residual ddfm=sat;
   random npsyllid;
   lsmeans host /adjust=tukey;
run;
proc mixed data=longevity order=data ;
   class host npsyllid;
   model ovi = host  /solution residual ddfm=sat;
   random npsyllid;
   lsmeans host /adjust=tukey;
run;
proc mixed data=longevity order=data ;
   class host npsyllid;
   model neggs = host  /solution residual ddfm=sat;
   random npsyllid;
   lsmeans host /adjust=tukey;
run;
  ODS GRAPHICS OFF; ODS HTML CLOSE;
```

11. Analysis ofadult sex ratio

```
ODS HTML; ODS GRAPHICS ON;
options nocenter nodate ls=90 ps=60;
data sexratio;
infile 'C:\JENITAC\AAAAA\SAS\nsexratio.csv' dlm=',' firstobs=3;
   input host $ group $ sex $ ratio;
  pratio = arsin(sqrt(ratio/100));
      run;
proc print;
run;
proc means mean stderr;
class host sex;
var ratio;
run;
proc mixed data=sexratio order=data ;
   class host group sex;
   model pratio = host sex /solution residual ddfm=sat;
   random group;
   lsmeans host sex/adjust=tukey;
run;
ODS GRAPHICS OFF; ODS HTML CLOSE;
```

12. Estimation of life table parameters

```
ods html;
options ls=70 nodate;
data ONE;
```

```
`


input GROUP $ NFEMALE $ AGE NEGGS SEXRATIO SURV;
cards;
POTHOT      1     18.4  0     0.4729729  0.9
.
.
.
POTCOLD     2     22.2  0     0.575 1.0
.
.
.
SLNHOT      1     23.4  0     0.60000    0.544554455
.
.
.
SLNCOLD     1     24.8  0     0.64  0.84211
.
.
.
;
/*-------------------------------------------------------------
-------------------------*/
/* Computation of the total number of eggs laid per female in
each group (NINDGR).       */
/*-------------------------------------------------------------
-------------------------*/

proc sort data=ONE;
        by GROUP NFEMALE AGE;

proc means data=ONE sum noprint;
        bY GROUP NFEMALE;
        var NEGGS;
        output out=FERT sum=TOTEGGS;

proc print data=FERT noobs;
title 'Total number of eggs laid per female in each group';
        by GROUP;
        var NFEMALE TOTEGGS;
run;

/*-------------------------------------------------------------
-------------------------*/
/* Computation of the number of individuals (females) in each
group (NINDGR).           */
/*-------------------------------------------------------------
-------------------------*/

proc sort data=ONE nodupkey out=TWO;
        by GROUP NFEMALE;
```

`

```
proc means noprint data=TWO;
        var NEGGS;
        by GROUP;
        output out=THREE n=NINDGR;

proc means noprint data=THREE;
        var NINDGR;
        output out=FOUR max=MAXNGR;

data _null_;
        set FOUR;
        call symput('N',MAXNGR);

data T;
        set THREE;
         do INDNUM=1 to &N;
          if INDNUM>NINDGR then delete;
          drop _type_ _freq_;
         output;
        end;

data T;
merge T TWO;
        by GROUP;

data ONESEQ;
merge ONE T;
        by GROUP NFEMALE;

proc sort data=ONESEQ;
        by INDNUM;

/*-----------------------------------------------------------------
-----------------------------------------------------------------
-------*/
/* Creation of a data set (SIX) with N+1 subsets (V), being the
first (V=0) with all females and */
/*  the N others with all minus one female. Different female is
dropped out in each step. This     */
/*  data set will be used for jackknife estimation.
*/
/*-----------------------------------------------------------------
-----------------------------------------------------------------
-------*/

data SIX;
set ONESEQ;
        V=0;
```

`

```
        %macro ALI;
        %do I=1 %to &N;
        %let G=&I;

data FIVE;
set ONESEQ;
        V=&G;
        if INDNUM=V then delete;

data SIX;
merge SIX FIVE;
        by V;
%end;
%mend ALI;
%ALI
run;

/*-------------------------------------------------------------------
-------------------------*/
/* Computation of the number of female eggs (NFEMEGGS) laid by
each female in each time  */
/* interval
*/
/*-------------------------------------------------------------------
-------------------------*/

proc sort data=SIX;
        by GROUP V INDNUM AGE;

data SIX;
set SIX;
        if V>NINDGR then delete;

proc sort data=SIX;
        by GROUP V AGE;

data SIX;
set SIX;
        NFEMEGGS=NEGGS*SEXRATIO;

proc means data=SIX noprint;
        id NINDGR SURV;
        by GROUP V AGE;
        var NFEMEGGS;
        output out=SEVEN mean=MX;

/*-------------------------------------------------------------------
-------------------------*/
```

`

```
/*     Computation of the net reproductive rate (RO)
*/
/*-------------------------------------------------------------
-------------------------*/

data EIGHT;
set SEVEN;
        NMATFEM= freq ;
        if V=0 then LX=SURV*(NMATFEM/NINDGR);
        if V>0 then LX=SURV*(NMATFEM/(NINDGR-1));
        LXMX=LX*MX;
        MXLXX=LXMX*AGE;
        drop _type_ _freq_;

proc print data=EIGHT noobs;
title 'Fertility lifetable';
        by GROUP;
        where V=0;
        var AGE SURV LX MX LXMX MXLXX;
run;

proc means noprint data=EIGHT;
        id NINDGR;
        by GROUP V;
        var LXMX MXLXX;
        output out=NINE sum=RO NUMT;

/*-------------------------------------------------------------
-------------------------*/
/* Estimation by iterative method of:mean generation time
(T),intrinsic rate of increase */
/* (RM), doubling time (DT) and finite rate of increase (LAMBDA)
*/
/*-------------------------------------------------------------
-------------------------*/

data TEN;
set NINE;
        T=NUMT/RO;
        RM=(LOG(RO))/T;
        keep GROUP NINDGR V RM RO;

data ELEVEN;
merge EIGHT TEN;
        by GROUP V;
        keep GROUP NINDGR V AGE RM RO LXMX;

data TWELVE;
set ELEVEN;
```

`

```
        do U=800 to 1200 by 5;
          R=RM*U/1000;
          Y=LXMX*(exp(-R*AGE));
          keep GROUP NINDGR V AGE Y R RO;
         output;
        end;

/*proc print data=ten noobs; /*
/*title 'Rm estimates obtained by using the approximate method';
*/
/*        where V=0; */
/*        var GROUP RM; */
/*run;  */

proc sort data=TWELVE;
        by GROUP V R;

proc means noprint data=TWELVE;
        by GROUP V R;
        var Y;
        id RO NINDGR;
        output out=THIRTEEN sum=SUM;

data FOURTEEN;
set THIRTEEN;
        DELTA=abs(1-SUM);
        drop _type_ _freq_;

proc means noprint data=FOURTEEN;
        by GROUP V;
        var DELTA;
        id R RO NINDGR;
output out=FIFTEEN min=DELTA;

data FIFTEEN;
set FIFTEEN;
        RM=R;
        DT=(log(2))/RM;
        T=(log(RO))/RM;
        LAMBDA=exp(RM);

data SIXTEEN;
set FIFTEEN; where V=0;
        RO0=RO;
        RM0=RM;
        T0=T;
        DT0=DT;
        LAMBDA0=LAMBDA;
        keep GROUP RO0 RM0 T0 DT0 LAMBDA0;
```

`

```
data SEVETEEN;
set FIFTEEN;
        where V>0;
        keep GROUP NINDGR V RO RM T DT LAMBDA;

data EIGHTEEN;
merge SIXTEEN SEVETEEN;
        by GROUP;

/*-------------------------------------------------------------
-------------------------*/
/*    Computation of the pseudo values for RO (ROPSV), RM
(RMPSV), T (TPSV), DT          */
/*     (DTPSV) and LAMBDA (LPSV)
*/
/*-------------------------------------------------------------
-------------------------*/

data NINETEEN;
set EIGHTEEN;
        ROPSV=(NINDGR*RO0)-((NINDGR-1)*RO);
        RMPSV=(NINDGR*RM0)-((NINDGR-1)*RM);
        TPSV=(NINDGR*T0)-((NINDGR-1)*T);
        DTPSV=(NINDGR*DT0)-((NINDGR-1)*DT);
        LPSV=(NINDGR*LAMBDA0)-((NINDGR-1)*LAMBDA);

proc print data=NINETEEN noobs;
title 'Parameter estimates using the whole data set - true
calculations';
        by GROUP;
        where V=1;
        var RO0 RM0 T0 DT0 LAMBDA0;
run;

/*-------------------------------------------------------------
-------------------------*/
/* Computation of the parameters 95% confidence limits using
jackknife estimates for the */
/* variance. The Student's t approximation is used.
*/
/*-------------------------------------------------------------
-------------------------*/

proc means noprint data=NINETEEN;
        id NINDGR;
        by GROUP;
        where V>0;
        var ROPSV RMPSV TPSV DTPSV LPSV;
```

`

```
        output out=TWENTY mean= RO_MEAN RM_MEAN T_MEAN DT_MEAN
L_MEAN
        lclm= RO_LLM RM_LLM T_LLM DT_LLM L_LLM
        uclm= RO_ULM RM_ULM T_ULM DT_ULM L_ULM
        var= RO_VAR RM_VAR T_VAR DT_VAR L_VAR;

proc print data=TWENTY noobs;
title 'Jackknife estimates for RO and respective confidence
limits';
        var GROUP RO_LLM RO_MEAN RO_ULM;

proc print data=TWENTY noobs;
title 'Jackknife estimates for RM and respective confidence
limits';
        var GROUP RM_LLM RM_MEAN RM_ULM;

proc print data=TWENTY noobs;
title 'Jackknife estimates for T and respective confidence
limits';
        var  GROUP T_LLM T_MEAN T_ULM;

proc print data=TWENTY noobs;
title 'Jackknife estimates for DT and respective confidence
limits';
        var  GROUP DT_LLM DT_MEAN DT_ULM;

proc print data=TWENTY noobs;
title 'Jackknife estimates for LAMBDA and respective confidence
limits';
        var GROUP L_LLM L_MEAN L_ULM;

/*-----------------------------------------------------------------
-----------------------------------------------------------------
-------*/
/* Creation of a data set (TFIVE) with all pairwise comparison
between groups. This data set will */
/* be necessary in t test application.
*/
/*-----------------------------------------------------------------
-----------------------------------------------------------------
-------*/

data _null_;
set TWENTY nobs=NGR;
call symput('NGR',NGR);

data T_ONE;
set TWENTY;
        GR=_n_;
```

`

```
        GR1=GR;
        GR2=GR;
        GRA=GROUP;
        GRB=GROUP;
        keep GROUP GR GRA GRB GR1 GR2 NINDGR RO_MEAN RM_MEAN
T_MEAN DT_MEAN L_MEAN
        RO_VAR RM_VAR T_VAR DT_VAR L_VAR;

data T_ONEA;
set T_ONE;
        if GR<&NGR;
        drop GR2 GRB;

data T_ONEB;
set T_ONE;
        if GR>1;
        drop GR1 GRA;

data T_TWO;
        F=&NGR-1;
        do GR1=1 to F;
         Z=GR1+1;
          do GR2=Z to &NGR;
          output;
         end;
        end;

data T_THREE;
merge T_TWO T_ONEA;
        by GR1;
        RO_MEAN1=RO_MEAN;
        RM_MEAN1=RM_MEAN;
        T_MEAN1=T_MEAN;
        DT_MEAN1=DT_MEAN;
        L_MEAN1=L_MEAN;
        RO_VAR1=RO_VAR;
        RM_VAR1=RM_VAR;
        T_VAR1=T_VAR;
        DT_VAR1=DT_VAR;
        L_VAR1=L_VAR;
        NINDGR1=NINDGR;
        keep GR1 GR2 GRA NINDGR1 RO_MEAN1 RM_MEAN1 T_MEAN1
         DT_MEAN1 L_MEAN1 RO_VAR1 RM_VAR1 T_VAR1
         DT_VAR1 L_VAR1;

proc sort data=T_TWO;
        by GR2 GR1;

proc sort data=T_THREE;
```

`

```
        by GR2;

data T_FOUR;
merge T_THREE T_ONEB;
        by GR2;
        RO_MEAN2=RO_MEAN;
        RO_VAR2=RO_VAR;
        RM_MEAN2=RM_MEAN;
        RM_VAR2=RM_VAR;
        T_MEAN2=T_MEAN;
        T_VAR2=T_VAR;
        DT_MEAN2=DT_MEAN;
        DT_VAR2=DT_VAR;
        L_MEAN2=L_MEAN;
        L_VAR2=L_VAR;
        NINDGR2=NINDGR;
        keep GR1 GR2 GRA GRB  NINDGR1 NINDGR2 RO_MEAN1 RM_MEAN1
T_MEAN1
        DT_MEAN1 L_MEAN1 RO_VAR1 RM_VAR1 T_VAR1 DT_VAR1 L_VAR1
RO_MEAN2
        RM_MEAN2 T_MEAN2 DT_MEAN2 L_MEAN2 RO_VAR2 RM_VAR2 T_VAR2
        DT_VAR2 L_VAR2;

proc sort data=T_FOUR;
        by GR1 GR2;

data T_FOUR;
set T_FOUR;
        N1=NINDGR1;
        N2=NINDGR2;

data T_FIVE;
set T_FOUR;
        PARAM='RO';
        MED1=RO_MEAN1;
        MED2=RO_MEAN2;
        VAR1=RO_VAR1;
        VAR2=RO_VAR2;
output;
        PARAM='RM';
        MED1=RM_MEAN1;
        MED2=RM_MEAN2;
        VAR1=RM_VAR1;
        VAR2=RM_VAR2;
output;
        PARAM='T';
        MED1=T_MEAN1;
        MED2=T_MEAN2;
        VAR1=T_VAR1;
```

```
`


        VAR2=T_VAR2;
output;
        PARAM='DT';
        MED1=DT_MEAN1;
        MED2=DT_MEAN2;
        VAR1=DT_VAR1;
        VAR2=DT_VAR2;
output;
        PARAM='L';
        MED1=L_MEAN1;
        MED2=L_MEAN2;
        VAR1=L_VAR1;
        VAR2=L_VAR2;
        keep GRA GRB N1 N2 PARAM MED1 VAR1 MED2 VAR2;
output;

proc sort data=T_FIVE;
        by PARAM;
/*-------------------------------------------------------------
-------------------------*/
/* The Student's t test for pairwise comparison of groups with
different variances. The  */
/* p values are calculated for bilateral (PBI), unilateral at
right (PR), and unilateral */
/* at left (PL) t test.
*/
/*-------------------------------------------------------------
-------------------------*/

data T_TEST;
set T_FIVE;
        STD1=sqrt(VAR1/N1);
        STD2=sqrt(VAR2/N2);
        DIF=MED1-MED2;
        T=DIF/(sqrt((VAR1/N1)+(VAR2/N2)));
        NUMDF= ((VAR1/N1)+(VAR2/N2))**2;
        DENDF=((VAR1/N1)**2)/(N1-1)+((VAR2/N2)**2)/(N2-1);
        DF=NUMDF/DENDF;
        PBI=2*(1-probt(abs(T),DF));
        PR=1-probt(T,DF);
        PL=1-probt((-1*T),DF);

proc print data=T_TEST noobs;
title2 'Student t-test for pairwise group comparison';
        var PARAM GRA GRB MED1 STD1 MED2 STD2 PBI PR PL T;
OPTIONS LS=80;
run;
```

`

```
/*-------------------------------------------------------------
-------------------------*/
/* Construction of graph showing oviposition pattern over time
in each group             */
/*-------------------------------------------------------------
-------------------------*/

proc sort data=ONE out=FERT2;
 by GROUP AGE;

proc means data=FERT2 noprint;
        var AGE;
        output out=FERT3 min=MIN max=MAX;

data _null_;
        set FERT3;
        MIN=MIN-0.5;
        MAX=MAX+0.5;
        call symput('AGEMIN' ,MIN);
        call symput('AGEMAX' ,MAX);


data FERT2;
 set FERT2;
 where NEGGS>0;

 label AGE= 'Female age at oviposition (days)';
 label GROUP= 'Group';
 label NEGGS= 'Number of eggs';

goptions reset=global gunit=pct
          ftext=simplex htext=4;

title 'Distribution of age at oviposition';

 symbol interpol=stdmjt   /* box plot              */
        cv=black          /* plot symbol color     */
        co=black          /* box and whisker color */
        width=1           /* line width            */
        value=dot         /* plot symbol           */
        height=1;         /* symbol height         */

 axis1 offset=(5,5)
       order=&AGEMIN to &AGEMAX by 7
       length=60
       minor=none;

 axis2 label=(angle=90 h=4)
        length=40
```

```
`


        major=(n=5)
        minor=none;

 proc gplot data=FERT2;
    by GROUP;
    plot NEGGS*AGE/haxis=axis1
                   vaxis=axis2;
  run;

/*-------------------------------------------------------------
-------------------------*/
/* Construction of box-plots for the number of eggs laid per
female in each group        */
/*-------------------------------------------------------------
-------------------------*/

data FERT;
 set FERT;
 label TOTEGGS= 'Total eggs laid per female';
 label GROUP= 'Group';

goptions reset=global gunit=pct
          ftext=simplex htext=4;

title 'Box and whisker plot of number of eggs laid per female';

 symbol interpol=boxtf05  /* box plot               */
        cv=grey           /* plot symbol color      */
        co=black          /* box and whisker color */
        width=1           /* line width             */
        value=dot         /* plot symbol            */
        height=2;         /* symbol height          */

 axis1 value=('Control' 'Treated')
       offset=(5,5)
       length=25;

 axis2 label=(angle=90)
       major=(n=5)
       minor=none
       length=35;

 proc gplot data=FERT;
    plot TOTEGGS*GROUP/haxis=axis1
                       vaxis=axis2;

/*-------------------------------------------------------------
-------------------------*/
```

`

```
/* Construction of box-plots for the pseudo-values of associated
life table parameters   */
/*-------------------------------------------------------------
-------------------------*/

data NINETEEN;
 set NINETEEN;
 label ROPSV= 'Ro pseudo-values (days)';
 label RMPSV= 'Rm pseudo-values (days)';
 label TPSV= 'T pseudo-values (days)';
 label DTPSV= 'Dt pseudo-values (days)';
 label LPSV= 'Lambda pseudo-values (days)';
 label GROUP= 'Group';

goptions reset=global gunit=pct
          ftext=simplex htext=4;

 symbol interpol=boxtf05  /* box plot              */
        cv=grey           /* plot symbol color     */
        co=black          /* box and whisker color */
        width=1           /* line width            */
        value=dot         /* plot symbol           */
        height=2;         /* symbol height         */

 axis1 value=('Control' 'Treated')
       offset=(5,5)
       length=25;

 axis2 label=(angle=90)
       major=(n=5)
       minor=none
       length=35;

 proc gplot data=NINETEEN;
 plot (ROPSV RMPSV TPSV DTPSV LPSV)*GROUP/haxis=axis1
                                          vaxis=axis2;

 run;
 quit;

ods html close;
```